ELIMINATION OF *LISTERIA MONOCYTOGENES* ON RAW CHICKEN FILLETS BY UV LIGHT WITHOUT INCREASING ANTIBIOTIC RESISTANCE AND AFFECTING MEAT COLOR

by

STEVEN ALTON LYON

(Under the Direction of Daniel L. Fletcher)

ABSTRACT

Raw chicken fillets were exposed to ultraviolet (UV) irradiation (dose of 1,000 μW/cm² for 5 min at a wavelength of 254 nm) to evaluate its potential to reduce *Listeria monocytogenes* on raw product before shipment to a further processing plant. Four strains were used based on differing subtypes and antibiotic resistance profiles. A 2 Log reduction in viable *L. monocytogenes* was observed with all strains for the UV irradiated fillets as compared to the non irradiated fillets. UV irradiation caused no significant changes in the antibiotic resistance profiles for all 4 strains with the UV irradiated fillets as compared to the non irradiated fillets. The UV irradiation treatment had no undesirable effects on meat color at day 0 and day 7 between the 2 groups. This thesis suggests that UV irradiation of raw fillets can significantly reduce *L. monocytogenes* without selecting for increased antibiotic resistance and negatively affecting meat color.

INDEX WORDS: ultraviolet irradiation, poultry processing, *Listeria monocytogenes*, food safety, antibiotic resistance, meat color
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DEDICATION

I would like to dedicate this thesis to my beloved family and closest friends. The goals that I have achieved, both personally and professionally, could not have been possible if not for my loving parents, Clyde Eugene and Brenda Gaston Lyon. Throughout all the years spent here at UGA, my mother and father were always there for encouragement, guidance, and support. I can’t thank them enough. I would also like to thank my closest friends and girlfriend, Kendra Bailey, who have meant so much to me for their support and encouragement when I needed it the most. I’ll never forget them nor will I never forget all the memories shared while downtown, tailgating, and watching the DAWGS win 2 SEC football championships, 3 SEC eastern division football championships, 2 Sugar Bowl trips, countless All-Americans and draftees into the NFL, 4 straight seasons in the football AP top 10, 2 Baseball College World Series appearances, 1 SEC baseball championship, 2 NCAA basketball tournament appearances, 1 SEC eastern division basketball championship, 3 SEC tennis championships, and 2 NCAA golf National Championships.
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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Table of Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Objectives</td>
<td>4</td>
</tr>
<tr>
<td>2 LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> Characteristics</td>
<td>5</td>
</tr>
<tr>
<td>Isolation and Identification from Foods and Processing Environments</td>
<td>7</td>
</tr>
<tr>
<td>Virulence Factors and Pathogenesis</td>
<td>9</td>
</tr>
<tr>
<td>Listeriosis and Sequelae Diseases</td>
<td>14</td>
</tr>
<tr>
<td>Antibiotic Treatment for Clinical Infections</td>
<td>19</td>
</tr>
<tr>
<td>Antibiotic Resistance</td>
<td>22</td>
</tr>
<tr>
<td>Antibiotic Resistance with <em>Listeria monocytogenes</em></td>
<td>30</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> and Integrated Poultry Production</td>
<td>35</td>
</tr>
<tr>
<td>Ultraviolet Irradiation</td>
<td>43</td>
</tr>
<tr>
<td>References</td>
<td>53</td>
</tr>
</tbody>
</table>
3 EFFECT OF GERMICIDAL ULTRAVIOLET LIGHT ON THE
ANTIBIOTIC RESISTANCE PROFILES OF LISTERIA
MONOCYTOGENES.................................................................69
Abstract .........................................................................................70
Introduction .....................................................................................71
Materials and Methods .....................................................................73
Results and Discussion ....................................................................78
References .........................................................................................81
Tables ..............................................................................................86

4 EFFECTS OF ULTRAVIOLET TREATMENT OF RAW CHICKEN
FILLETS ON THE RECOVERY OF LISTERIA MONOCYTOGENES
AND MEAT COLOR .......................................................................92
Abstract ........................................................................................93
Introduction .....................................................................................94
Materials and Methods ....................................................................96
Results and Discussion ...................................................................100
References .......................................................................................106
Tables ..............................................................................................110

5 SUMMARY AND CONCLUSION ......................................................112
LIST OF TABLES

Table 3.1: Antibiotic resistance profiles of *Listeria monocytogenes* isolates prior to exposure to UV irradiation.................................................................86

Table 3.2: Antibiotics and extent of dilutions in GPN2F panels for *Listeria monocytogenes* including breakpoint criteria in terms of minimal inhibitory concentration (MIC)...........................................................................................................87

Table 3.3: Number and percentage of intermediate / resistant isolates from 161 *Listeria monocytogenes* isolates recovered from a poultry further processing plant......89

Table 3.4: Number and percentage intermediate / resistant *Listeria monocytogenes* isolates for control groups and UV treatment groups........................................91

Table 4.1: Mean and standard error of the mean Log_{10} CFU / breast values of 4 strains of *Listeria monocytogenes* for UV treated and untreated breast fillets ..........110

Table 4.2: L*, a*, and b* color space values (mean ± std error) of UV treated and untreated raw skinless boneless chicken breast fillets.................................111
CHAPTER 1
INTRODUCTION

A major goal for both poultry processors and the USDA Food Safety Inspection Service is to supply consumers with safe food. The Centers for Disease Control (CDC) estimates that 76,000,000 cases of foodborne illnesses occur in the United States every year that result in 300,000 hospitalizations and 5,000 deaths (Anonymous, 2003). Consumption of contaminated poultry products are often the cause for some of these diseases. *Listeria monocytogenes* has become a persistent threat of concern to poultry further processors and to immunocompromised consumers. Although estimated cases of listeriosis (2,500) are far fewer than those of *Campylobacter* and *Salmonella* spp., this bacterium is responsible for about 500 deaths per year in the United States (Anonymous, 2005a).

*L. monocytogenes* is widely distributed in nature and often ends up in poultry production. Unlike *Campylobacter* and *Salmonella* spp., *L. monocytogenes* is not a raw poultry safety concern but is more of a concern when it contaminates cooked poultry products. However, raw poultry meat, coming into a further processing plant from a slaughter plant, has been directly linked to the contamination of the further processing facility (Berrang et al., 2005). Depending on its genotypic characteristics, some strains of *L. monocytogenes* are able to persist and establish dominance in the further processing plant environment (Lemaitre et al., 1998; Autio et al., 2003).
Due to *L. monocytogenes*’ ability to survive in harsh environments (Fenlon, 1999), it can be very difficult to control in a poultry further processing plant. A loss of process control can result in the contamination of cooked poultry.

Listeriosis is a foodborne disease caused by the consumption of foods contaminated with *L. monocytogenes*. Individuals with underlying conditions such as decreased T-cell mediated immunity are at the highest risk of acquiring listeriosis (Mackaness, 1962). Although mostly sporadic, listeriosis has a mortality rate of 20% or higher (Slutsker and Schchat, 1999) which is greater than the mortality rates of *Campylobacter* and *Salmonella* spp. combined. There are two types of listeriosis, invasive and noninvasive. Noninvasive listeriosis results in mild flu like symptoms in healthy individuals while invasive listeriosis most often affects the immunodeficient. Invasive listeriosis often results in fatal secondary diseases such as severe meningitis, septicemia, primary bacteremia, endocarditis, and nonmeningitic central nervous system infection (Donnelly, 2001). Pregnant women are also highly susceptible; listeriosis can cause them to have stillbirths or spontaneous abortions of the developing fetus.

A successful processing method used to disinfect liquids is Ultraviolet (UV) irradiation. UV energy has a long history as an antimicrobial and has shown to also be effective at reducing bacteria on fresh meats (Stermer et al., 1987; Wallner-Pendleton et al., 1994; Sumner et al., 1996). UV has a bactericidal effect because it damages the DNA double helix and membrane proteins. As a result of UV absorption, both DNA replication and transcription are blocked and the cell dies if it cannot recover (Guerrero-Beltran and Barbosa-Canovas, 2004; Lado and Yousef, 2002).
Another advantage of using UV light as a processing aid is that it is a nonthermal process that does not negatively affect color or rancidity of fresh raw poultry meat (Wallner-Pendelton, et al., 1994).

UV disinfection of wastewater effluent has been shown to increase the antibiotic resistance profiles of coliforms (Meckes, 1982) and fecal coliforms (Staley et al., 1988) post UV treatment. Meckes (1982) also noted that the percentage of antibiotic resistance transfer to a donor increased from 43% to 49% post UV treatment. Currently, antibiotic resistance is low in *L. monocytogenes* as compared to other poultry associated pathogens. However, if UV exposure does select for highly resistant or multi-drug resistant survivors, the process could be more detrimental than beneficial. A potential problem to both processors and consumers of poultry products would be if UV irradiation selected for ampicillin, gentamicin, vancomycin, and or trimethoprim / sulphamethoxazole resistant *L. monocytogenes* survivors. Resistances to these drugs could result in untreatable listeriosis infections.

Therefore, it is hypothesized that UV irradiation of raw boneless skinless chicken breasts immediately following deboneing at the raw processing plant could eliminate *L. monocytogenes* prior to their shipment to a further processing plant. Since raw poultry is a vector for *L. monocytogenes* entering a poultry further processing plant, its elimination, and along with proper sanitation programs, could decrease the presence and persistence of this pathogen in further processing plants. However, if UV irradiation selects for multi-drug resistant *L. monocytogenes* survivors, then the presence and persistence of these strains in a poultry further processing plant could lead to the contamination of cooked poultry products and to the emergence of untreatable listeriosis infections.
Objectives

The objectives of this thesis were to determine the effects of ultraviolet irradiation applied to raw chicken fillets prior to arrival at a poultry further processing plant:

a) To evaluate the effect of UV irradiation of raw chicken breast filets on the recovery of *Listeria monocytogenes*.

b) To determine if UV irradiation changed the antibiotic resistance profiles of surviving *Listeria monocytogenes*.

c) To determine if any undesirable color changes occurred on the meat due to UV irradiation.
CHAPTER 2

LITERATURE REVIEW

Listeria monocytogenes Characteristics

Listeria monocytogenes is a small (1.0-2.0 x 0.5 µm), gram positive, non-
sporeforming, rod shaped bacterium. It is an intracellular opportunistic pathogen that can
survive and grow within monocytes and neutrophils. Thus, it was named because of the
large numbers of monocytes commonly found in the peripheral blood of its hosts after an
infection (Gray and Killinger, 1966). Despite the inability to form spores, L.
monocytogenes is a remarkably hardy microorganism capable of surviving and adapting
to a variety of stresses. It can survive freezing and grow in the temperature range of 1°C
to 50°C, pH range of 4.0 – 9.5, and water activity range of 0.90 – 0.97 (Martin and
Fisher, 1999). The bacterium is capable of forming biofilms after attachment to a variety
of surfaces. It is motile by peritrichous flagella in their natural environments at ambient
temperatures. L. monocytogenes can also be characterized as a facultative anaerobe that
is catalase-positive and oxidase-negative. Cells are typically rods found alone, in short
chains, or can be arranged in V and Y forms (Rocourt, 1999). Cells can sometimes
appear coccoid and be mistaken for Staphylococcus spp. Old cultures may also lose their
purple stain during Gram staining (Rocourt, 1999).

The natural habitats for L. monocytogenes are soil, water, silage, sewage, and
decaying plant material. Habitats important to food safety include the gastrointestinal
tracts of animals, asymptomatic human carriers, and food processing facilities. However, since *L. monocytogenes* is ubiquitous, it is very difficult to prevent it from contaminating food products. *L. monocytogenes* has better ability to survive under adverse environmental conditions than many other non-sporeforming bacteria of importance in foodborne disease (Fenlon, 1999). Since *L. monocytogenes* has the ability to colonize, multiply, and persist on processing equipment, it is a serious threat to the food industry (Fenlon, 1999).

*L. monocytogenes* is capable of adapting to and surviving in hazardous conditions like heat and acid stress. Once faced with these stresses, the bacterium enters a “survival mode”. When environmental stress occurs, shock proteins are synthesized by the regulation of sigma factors. Sigma factors are common genetic regulatory factors that are involved in enhancing stress resistance by binding to core microbial RNA polymerase and expressing shock protein synthesis (M. A. Harrison, University of Georgia, Athens, GA, personal communication). Two types of shock proteins produced by *L. monocytogenes* are heat shock proteins (HSP) and acid shock proteins (ASP). Shock proteins protect intracellular and extracellular proteins by preventing unfolding and subsequent denaturization by heat and or acid stress. Both HSP and ASP are synthesized in acute amounts under favorable conditions, but increase production immediately under stressful conditions and then gradually decrease production when favorable conditions return.

*L. monocytogenes* induces an acid tolerance response (ATR) when subjected to unfavorable acidic conditions. The pathogen uses the AR2 system which requires glucose and glutamine through the expression of *gadA* and *gadB*. *L. monocytogenes*
cells exposed to pH 5.5 for 30 minutes can result in the subsequent survival of these cells to a lethal pH of 3.5 (Hill and Gahan, 2000). The ATR makes the bacterium capable of surviving lethal pH conditions following adaptation to sublethal pH levels. The ATR is also capable of providing *L. monocytogenes* cross protection against lethal doses of hydrogen peroxide, heat, NaCl, ethanol, certain hydrophobic compounds, and other antimicrobials. Acid tolerant *L. monocytogenes* mutants have increased virulence compared to parental cells (O’Driscoll et al., 1996).

*L. monocytogenes* produce HSP when temperatures are greater than that for optimum growth and make the bacterium thermotolerant. HSP protect the bacterium against surface and internal protein damage. In addition, HSP assist in the refolding of damaged proteins as well as assisting RNA transcription, DNA replication, and flagella synthesis (M. A. Harrison, University of Georgia, Athens, GA, personal communication).

**Isolation and Identification from Foods and Processing Environments**

The Food Safety Inspection Service (FSIS) of the USDA has developed a procedure for the isolation and identification of *L. monocytogenes* involving an enrichment broth. Enrichment broths are necessary because they provide nutrients for cells, help injured cells recover, and contain various antimicrobials that inhibit background flora so that *L. monocytogenes* is more readily recovered (Ryser and Donnelly, 2001). The antimicrobials which *L. monocytogenes* are resistant to most often include nalidixic acid, cyclohexamide, and acriflavin (Ryser and Donnelly, 2001).
The standard USDA-FSIS isolation protocol (Johnson, 1998) was established for raw meat and poultry products and requires that a 25-g food sample be placed in a sterile bag with 225 ml of University of Vermont (UVM) modified enrichment broth and stomached for 2 minutes. After incubation at 30°C for 24 hours, a 0.1 ml portion is transferred to 10 ml of Fraser broth. If the Fraser broth tubes are black after an incubation period of 24 to 48 hours at 37°C, they are streaked onto modified oxford (MOX) selective agar plates and incubated for 24 to 48 hours at 37°C. MOX agar is used because it supplies nutrients for growth and antimicrobials to decrease background bacteria.

The growth rate for *L. monocytogenes* on culture media is increased by the presence of fermentable sugars, particularly glucose (Rocourt, 1999). Other growth requirements include the amino acids cystine, leucine, isoleucine, arginine, methionine, valine and cysteine, and the B vitamins riboflavin, biotin, thiamine, and thioctic acid (Martin and Fisher, 1999). On MOX agar, typical *L. monocytogenes* colonies appear small (1 – 2 mm) in diameter, grayish in color, and are surrounded by a black halo caused by esculin hydrolysis (Wallace et al., 2003). Colonies may be smooth or rough. Characteristic colonies then undergo further testing to completely distinguish *L. monocytogenes* from other *Listeria* spp. Gram staining results in purple uptake since *L. monocytogenes* are Gram-positive. Presumptive colonies are streaked onto Columbia base agar (CBA) with sheep blood overlay for the β-hemolysis test. A complete zone of hemolysis will surround colonies of *L. monocytogenes* and *L. innocua*. To differentiate between these 2 organisms, a Christie-Atkins-Munch-Peterson (CAMP) test should be performed. A disc containing *Staphylococcus aureus* is placed in the center of a sheep
blood agar plate. Presumptive colonies are streaked in a straight line from the outside of the plate towards and ending near the disc. The plate is then incubated for 24 hours at 37°C. Positive \textit{L. monocytogenes} colonies have an enhanced zone of β-hemolysis influenced by \textit{S. aureus} (Martin and Fisher, 1999). Other tests include the production of acids from sugars. Three sugars, mannitol, rhamnose, and xylose are used to distinguish possible \textit{L. monocytogenes} cultures. \textit{L. monocytogenes} produces acid only in rhamnose. Other tests include a catalase test, wet mount for tumbling motility, and umbrella motility.

Food processing machinery and the processing environment are important places to sample because \textit{L. monocytogenes} might be present. According to Ryser and Donnelly (2001), surface swabbing with a sterile 3” x 3” sponge or moistened gauze should be used. Sponges or gauze should then be placed in 200 ml of UVM broth, stomached for 2 minutes and incubated for 24 hours at 30°C (Ryser and Donnelly, 2001). The rest of the procedure is identical to the USDA-FSIS procedure.

\textit{Virulence Factors and Pathogenesis}

Virulence is the ability of a bacterium to cause disease. Virulence factors are bacterial products or strategies that contribute either to virulence or to pathogenicity. Virulence factors have two categories: those that promote bacterial colonization and those that cause damage to the host. Although not all strains of \textit{L. monocytogenes} are virulent, those that are have both of these factors (Salyers and Whitt, 2002). Most of \textit{L. monocytogenes} virulence genes are located in the same region on the chromosome in the
PrfA dependent virulence gene cluster (Kuhn and Goebel, 1999). PrfA may respond to temperature and acid stress thus, making stress adapted mutants more virulent.

*L. monocytogenes* has the following virulence factors; ATR, internalins (invasins), listeriolyisin O toxin (LLO), actin rocket polymerization (ActA), phospholipases (*plcA* and *plcB*), metalloprotease, and biofilm formation.

The ATR is vital for *L. monocytogenes* to survive gastric pH of < 2 and still be viable to cause infections. Through emergency pH homeostasis, the internal pH remains near neutral while the external pH is near 2. The ATR is an important factor while trapped in the endosomes of non phagocytic cells and in the phagosomes of monocytes, neutrophils, and macrophages. Once inside a host cell endosome or phagosome, the pH declines to 5.5. This would normally stop metabolic activity of other pathogens but this pH decline activates the expression for the listeriolyisin O toxin.

Motility may or may not be a direct virulence factor. Since *L. monocytogenes* must be ingested, it must survive passage through the stomach and then find and adhere to the mucosa of the small intestine to cause an infection. However, flagella expression occurs at temperatures lower than that of the human body (20 - 25°C). Thus, at 37°C production of flagella ceases (Schlech, 1988). It is possible, however, that ingested cells that came from an environment below 25°C may still have flagella for sometime after they reach the small intestine. If this is the case, flagella could help it reach and navigate through the intestinal mucosa (Salyers and Whitt, 2002).

Since *L. monocytogenes* is an intracellular pathogen it must adhere to the host cell membrane and then invade that cell. *L. monocytogenes* employs the “zipper mechanism” to invade non phagocytic host cells. The intestinal enterocytes are the first host cells to be
invaded. Internalins (IlnA and IlnB) are invasin molecules on the cell surface of *Listeria monocytogenes* and mediate adherence and invasion. IlnA, the primary invasion molecule, binds to E-cadherin on the surface of enterocytes (Martin and Fisher, 1999). IlnB can assist IlnA in attachment but its presence is not required. Once the internalins attach to the host cell, a cytoskeletal change occurs inside the host cell. The rearrangement of the host cell actin filaments causes forced phagocytosis of the bacterium. At this point, *L. monocytogenes* is inside a host cell endosome.

Listeriolysin O (LLO) is a pore forming, sulphydryl-activated hemolytic cytotoxin expressed by the *hly* gene. The presence of LLO causes the zone of beta-hemolysis on blood agar plates and is the most important virulence factor for a strain to be pathogenic (Salyers and Whitt, 2002). *L. monocytogenes* must escape the vesicles in which they are trapped to in order to grow in the host cytoplasm. The expression of the LLO toxin is due to the drop in pH and increase in temperature once enclosed within an endosome or phagosome. LLO is secreted and the toxin attaches itself to the cholesterol receptors on the membrane of the endosome or phagosome. The pore forming protein degrades the membrane of the endosome or phagosome. Along with LLO, *L. monocytogenes* uses two lipases. Phosphatidylinositol-specific phospholipase C (PI-PLC), encoded by the *plcA* gene, is an enzyme that hydrolyzes phosphatidylinositol in the membrane and contributes to vacuole escape in cells such as bone-marrow-derived macrophages. Phosphatidylcholine-specific phospholipase C (PC-PLC), encoded by the *plcB* gene, works with metalloprotease to enable listeriolysin O-independent escape of *L. monocytogenes* from primary vacuoles in human epithelial cells. PC-PLC has the ability to cleave most phospholipids. Once the vesicle membrane is dissolved, *L.
*L. monocytogenes* enters the cytoplasm where it can grow. *Hly* mutants, unable to express LLO, are not able to escape the endosome of non phagocytic cells and cannot replicate in the cytoplasm. Virulence for *L. monocytogenes* is based on the ability to survive and grow inside the host cells (Salyers and Whitt, 2002).

Once inside an infected host cell, *L. monocytogenes* uses the host cell’s actin filaments to move within and between adjacent host cells (Salyers and Whitt, 2002). *L. monocytogenes*, like *Shigella dysenteriae*, is capable of organizing host cell actin filaments into long tails that propel the bacterium through the host cell cytoplasm. This phenomenon is termed “actin rocket polymerization”, and this process requires the ActA protein synthesized from the *acta* gene (Martin and Fisher, 1999). ActA are surface proteins that attract actin filaments to the surface of *L. monocytogenes*. Actin filaments form an “actin cloud” that surrounds the bacterium. After the bacterium divides into two cells, the actin polymerization moves to the old ends of the bacterium. This results in the actin tails being present at only one end on the bacterium’s surface. Therefore, their movement through the cytoplasm is in one direction. *L. monocytogenes* can move rapidly through the cytoplasm of host cells by actin rocket polymerization. Salyers and Whitt (2002) estimate *L. monocytogenes* can move at a rate of about 1.5µm/s.

As *L. monocytogenes* moves to adjacent host cells it must pass through the cell membrane of the infected cell and the adjacent cell. The force of the propulsion allows the bacterium to penetrate both cell membranes (Martin and Fisher, 1999). Although cell membranes of the host cell are destroyed, the moving bacterium is surrounded by both membranes. Once inside this double membrane-bound vacuole, *L. monocytogenes* again has to escape into the cytoplasm.
PC-PLC plays a direct role in the degradation of escaping these lipid barriers through lipase activity. PI-PLC and metalloprotease also play roles in forming plaques in these membranes.

There are advantages to *L. monocytogenes* once it becomes intracellular. Essential nutrients from the host cell are available to the bacterium. *L. monocytogenes* can multiply rapidly in the host cytoplasm dividing once every 50 minutes (Salyers and Whitt, 2002). After the epithelium layer has been destroyed, the bacterium can move deeper through the submucosa layers and eventually into the blood stream. Once in the circulatory system, the infection can become systemic. A major advantage to the bacterium is that it is safe from most of the host’s defense systems. The Humoral Immune System, or complement cascade system, is ineffective against intracellular pathogens. Complement proteins cannot enter the host cell and cause opsonization. Antibodies of the Acquired Immune System are also helpless because they only work outside the host cell. Monocytes, neutrophils, and macrophages are not capable of killing the pathogen once inside a host cell. The bacteria are not fully protected however, because cytotoxic T lymphocytes are capable of killing the infected cell and *L. monocytogenes* inside that cell by apoptosis.

Biofilms may be another virulence factor of *L. monocytogenes*. A biofilm is a community of cells and their cellular products that are formed after the bacteria have attached to a surface, such as stainless steel equipment in a poultry processing plant (M. E. Berrang, USDA, Athens, GA, personal communication). This film consists of a community of many species of bacteria that are capable of communicating and exchanging genes with each other. The exchange of antibiotic resistance genes occurs
frequently in biofilms. The barrier can be difficult to remove by chemicals and by physical means such as scrubbing. The viscous coating itself also increases the resistance to antimicrobials. Frank and Koffi (1990) discovered that \emph{L. monocytogenes} had various resistances to antimicrobials and heat after initial attachment to a surface due to the production of surface-bound lipopolysaccharide-like substances. Sanitizers, ethanol, chlorines, and other antimicrobials have difficulty penetrating the thick matrix of a biofilm. Biofilms give the bacterial community resistance to many environmental and medical stresses. They also give the bacterial community persistence in inhabiting a niche either in a food processing environment or the host’s body.

\textbf{Listeriosis and Sequelae Diseases}

Although there are 5 other species in the genus \emph{Listeria}, \emph{L. monocytogenes} is the only species that is capable of causing disease in humans (Donnelly, 2001). \emph{L. monocytogenes} has been recognized as a foodborne pathogen since 1929 and causes listeriosis which can become epidemic. Since the bacterium is ubiquitous in the environment, it can frequently come into contact with both animals and humans (Marth, 1988). However, exposure does not always result in infection (Marth, 1988). Donnelly (2001) estimates that 5\% of healthy humans may harbor \emph{L. monocytogenes} in their gastrointestinal tract. Listeriosis is recognized as a rare but often fatal illness with a mortality rate of approximately 20\% or higher in susceptible individuals (Slutsker and Schuchat, 1999). The immunocompromised including pregnant women are at the highest risk of infection.
Although the incidence of *L. monocytogenes* infections is low, the death rate is much higher than other food pathogens such as *Salmonella* spp., *Escherichia coli O157:H7*, and *Campylobacter* spp. (Anonymous, 2005a).

In adults, listeriosis is divided into two primary syndromes, an invasive form and a noninvasive form. The noninvasive form of listeriosis may cause febrile gastroenteritis and mild flu like symptoms in healthy individuals. Symptoms include fever, fatigue, malaise, headache, nausea, cramps, vomiting, and diarrhea. The median incubation period of the noninvasive form of listeriosis is 18 – 20 hours (Donnelly, 2001).

The invasive form of listeriosis has a much greater mean incubation period of 31 days (Donnelly, 2001), and a lower infectious dose is needed to cause serious illness or death in immunocompromised and pregnant individuals (Dalton et al., 1997). Disease symptoms of the invasive form are more serious and include severe meningitis, septicemia, primary bacteremia, endocarditis, nonmeningitic central nervous system infection, conjunctivitis, and the febrile gastroenteritis (Donnelly, 2001). The invasion and intercellular spread of *L. monocytogenes* allows it to penetrate many tissue barriers. Two of the most important barriers penetrated are through the placenta, from the mother to the fetus, and the blood brain barrier, enabling access to the meninges.

Susceptible individuals typically have one or more underlying conditions that predispose these patients to acquiring invasive listeriosis. Those with the highest risk include pregnant women, fetuses, neonates, the elderly, organ transplant patients, and those receiving immunosuppressive therapy such as corticosteroids and antimetabolites. Also, people with alcoholism, diabetes, heart disease, or AIDS are at a high risk for invasive listeriosis (Martin and Fisher, 1999).
T cell-mediated immunity plays a major role in determining the resistance or susceptibility of a human host to an infection by *L. monocytogenes* (Mackaness, 1962). Cell mediated immunity depends on the phagocytic monocytes being an early response system and nonspecific effectors and T lymphocytes are a secondary response system used by the human host to stop infections (Martin and Fisher, 1999; Donnelly, 2001). Weakened and or altered monocytes, neutrophils, and T cells impair the cell-mediated immunity allowing a *Listeria* infection to occur after initial exposure.

Pregnant women and their developing fetuses are at high risk of invasive listeriosis and are often instructed not to eat deli meat, certain cheeses, and unpasteurized foods due to the possible exposure to *L. monocytogenes*. During gestation, cell-mediated immunity becomes depressed as a means of preventing the rejection of the fetus by the mother (Donnelly, 2001). This reduction decreases the maternal resistance to *L. monocytogenes* infections and increases the risk of invasive listeriosis (Martin and Fisher, 1999). Levels of hormones and serum factors that produce and activate macrophages are decreased. Hydrocortisone plasma levels may increase up to seven times higher than that of a nonpregnant woman which suppresses the activation of lymphokines and the phagocytosis of macrophages (Weinberg, 1984). Low levels of immunoglobulin M (IgM) and decreased activity of classic complement pathway during the neonatal period also occur and demonstrate the importance of opsonization in the immune response to *L. monocytogenes* (Gellin and Broome, 1989).

During the third trimester of gestation, the fetus is the most susceptible to listeriosis (Marth, 1988; Martin and Fisher, 1999; Donnelly, 2001). There are three outcomes due to the infection of a pregnant woman to invasive listeriosis. One, the
mother may be asymptomatic but the developing fetus will become infected. Two, the mother will become severely ill and enter premature labor. Stillbirth or spontaneous abortion could occur. Finally, although rare, the fetus is unaffected but the mother dies. In most cases, the mother experiences mild flu like symptoms. However, neonatal fatality and morbidity are common.

There are two types of listeriosis with neonates; early and late onset (Martin and Fisher, 1999). Neonatal listeriosis is often fatal due to bacteremia or septicemia (Marth, 1988). In early onset listeriosis, *L. monocytogenes* crosses the placenta to cause the infection of the fetus (Marth, 1988). This syndrome is known as granulomatosis infantisepticum characterized by necrosis of the internal organs, particularly the liver, lungs, kidneys, and brain (Martin and Fisher, 1999). Early-onset listeriosis of neonates usually results in stillbirth or spontaneous abortion of the fetus. If the fetus is born alive, recovery from brain damage is highly unlikely. Late-onset listeriosis can occur from several days to two weeks after a full term birth when the babies may appear to be healthy. The mother usually has no complications with her pregnancy. Sources of *L. monocytogenes* to pregnant women are unknown but could be due to a nosocomial infection. Neonatal meningitis is the most common disease to the babies with late-onset listeriosis (Martin and Fisher, 1999).

There is strong evidence from outbreaks of listeriosis that the primary mode of transmission of *L. monocytogenes* from the environment to humans is through the consumption of contaminated foods (Schlech, 1988). Recent outbreaks of listeriosis have involved the most predominant serotype in the United States and Canada; 4b (Schlech, 1988., Martin and Fisher, 1999). The infectious dose required to cause listeriosis
depends on the virulence of the strain and the immunity of the host. Martin and Fisher (1999) believe that ingesting less than 1,000 cells could cause the disease.

Although most outbreaks of listeriosis are sporadic illnesses, epidemic outbreaks have occurred with dairy and poultry products. In 1985, the deadliest of all known foodborne disease outbreaks in the United States occurred with *L. monocytogenes* in southern California (Ryser, 1999). The consumption of contaminated Mexican-style cheese made *L. monocytogenes* an important foodborne pathogen and thus, the federal government began to regulate *L. monocytogenes* in food processing plants. In this outbreak, 300 cases were reported and 85 (28%) deaths occurred. It was determined that the source was illegally processed cheese that was made with raw milk in combination with pasteurized milk. The causative serotype, 4b, was matched with isolates from the cheese processing plant and growth on the final product. A Class I recall was established and the company shut down. In all, 250 tons of cheese products were buried (Ryser, 1999).

The first case of human listeriosis from a ready-to-eat poultry product was in 1989 from the consumption of contaminated turkey franks (Wenger et al., 1990). The causative serotype was 1/2a. The serotype was later matched to uneaten franks from the infected person’s refrigerator and from the conveyor belt attached to the peeler machine in the processing plant. Contaminated hot dogs were also the cause of a listeriosis outbreak from a Sara Lee Corporation processing plant (Anonymous, 1999a). In that outbreak, 4b was responsible for 101 cases of listeriosis that resulted in 15 deaths and 6 miscarriages. The federal government believed the outbreak occurred because of the
removal of a large ventilation unit near the hot dog packaging line that allowed airborne
*L. monocytogenes* to contaminate the equipment (Anonymous, 1999a).

The biggest outbreak of listeriosis involving poultry was in 2002 in the northeastern United States (Anonymous, 2002). Wampler Foods in Pennsylvania voluntarily recalled 27.4 million pounds of processed poultry, the largest ever recall of meat. As a result of consuming *L. monocytogenes* contaminated processed chicken and turkey deli meat, 46 confirmed cases of listeriosis were reported. Seven people died and 3 stillbirths or abortions occurred. *L. monocytogenes* was recovered from 25 environmental samples and 2 cooked turkey products. The strains recovered from cooked turkey were different from the strain(s) responsible for the outbreak. However, two samples from the floor drains were determined to be indistinguishable by the CDC from strains isolated from the infected patients.

**Antibiotic Treatment for Clinical Infections**

Antibiotics are naturally occurring low molecular weight compounds that either kill or inhibit the growth of bacteria. Antibiotics target features in prokaryotic cells that are not found in eukaryotic cells. They also may target enzymes or metabolic pathways that are essential for the bacteria to grow. In the case of listeriosis, it is paramount that the patient receives antibiotic therapy as soon as possible (Marth, 1988). If antibiotics are administered too late, the patient may never have a full recovery. Antibiotic therapy for listeriosis can last for 6 weeks. Immunosuppressed patients require a 3 – 6 week period of antibiotic therapy because their body cannot clear infected host cells (Armstrong, 1995). Slutsker and Schuchat (1999) state that a proper treatment course of antibiotic
therapy could be 2 weeks for pregnant women, 2 – 3 weeks for neonates, 2 – 4 weeks for healthy individuals with meningitis, bacteremia, and endocarditis.

The drugs of choice are ampicillin alone or in combination with an aminoglycoside such as streptomycin or gentamicin (Charpentier and Courvalin, 1999). Penicillin G (Pfizerpen) with an aminoglycoside is another option. Ampicillin and penicillins are β-lactam antibiotics. β-lactams derive their name from the four-membered ring this class of antibiotics all have in common (Salyers and Whitt, 2002). β-lactams bactericidal effect is due to inhibition of the final stage in peptidoglycan (cell wall) synthesis (Rusin and Gerba, 2001). Transpeptidase, or penicillin binding protein, is inhibited from cross-linking the peptides that form the chains of the growing peptidoglycan layer. Inhibition of this enzyme occurs due to the similarity in structure of β-lactams and D-ala links on the base of these chains. Transpeptidase binds to β-lactams instead of D-ala and the reaction of cross-linking chains does not occur. Besides the inability to grow, another result of β-lactams inhibition of cross-linking is the triggering of endogenous enzymes that decompose the peptidoglycan layer (Salyers and Whitt, 2002). These enzymes are normally involved in the turnover (recycling) of peptidoglycan so the cell can grow and divide (Salyers and Whitt, 2002). However, β-lactams control them to stimulate an attack on the peptidoglycan layer (Salyers and Whitt, 2002). The peptidoglycan layer protects the cell from bursting during exposure to different osmotic concentrations within and outside the cell (Salyers and Whitt, 2002). Therefore, its destruction can lead to bacterial cell lysis.
β-lactams differ greatly in toxicity, stability, administration, time of clearance from the bloodstream, and the ability to cross the blood brain barrier which is important for listeriosis infections (Salyers and Whitt, 2002).

There are disadvantages to using β-lactams with listeriosis infections. *L. monocytogenes* usually has a very slow response to them. Slutsker and Schuchat (1999) state that β-lactams in general are poor at penetrating into host cells. Thus, the ability of *L. monocytogenes* to grow and survive within cells probably explains the poor response to penicillin (Southwick and Purich, 1996) and the need to use it with an aminoglycoside. Intracellular concentrations of penicillin are usually insufficient to eradicate *L. monocytogenes* (Slutsker and Schuchat, 1999). Also, there are many people who are allergic to β-lactams. An allergic reaction can occur due to the formation of β-lactam/serum protein conjugate which can evoke an immune response in susceptible individuals (Salyers and Whitt, 2002).

Aminoglycosides are trisaccharides with amino groups that act on the 30S portion of the bacterial ribosome (Salyers and Whitt, 2002). Bacterial ribosomes are an excellent target because they are different from mammalian ribosomes (Salyers and Whitt, 2002). This class of antibiotics changes the shape of the 30S portion, causing the code on mRNA to be read incorrectly (Rusin and Gerba, 2001). The incorrect coding inhibits protein synthesis. This action makes aminoglycosides bactericidal because essential protein synthesis is inhibited. Unlike β-lactams, aminoglycosides can be taken up by host cells and this is the reason that the two drugs are prescribed together (Slutsker and Schuchat, 1999). Aminoglycosides do have side effects that limit their use. Prolonged use of these antibiotics may cause kidney damage or lead to deafness (Salyers and Whitt, 2002).
If a listeriosis patient is allergic to β-lactams, the second line drugs of choice are trimethoprim and sulphamethoxazole (Poyart-Salmeron et al., 1990). Trimethoprim and sulphamethoxazole are two antimetabolic drugs used in tandem to affect bacterial growth by inhibiting the tetrahydrofolate pathway (Salyers and Whitt, 2002). Tetrahydrofolic acid is an essential cofactor for bacteria to synthesize nucleic acids. Sulphamethoxazole resembles p-aminobenzoic acid in structure and is thus able to inhibit the action of the first enzyme in the tetrahydrofolate pathway, dihydropteroate synthase (Salyers and Whitt, 2002). Trimethoprim structure resembles dihydrofolic acid and it inhibits the enzyme dihydrofolate reductase (Salyers and Whitt, 2002). Although these two antimetabolites block different reactions of the same the pathway, they must be used together to be most effective. Trimethoprim and sulphamethoxazole have several therapeutic advantages over β-lactams and aminoglycosides. First, they don’t harm host cells because humans don’t have a folic acid synthesis pathway (Salyers and Whitt, 2002). Instead, humans require folate in their diets. Second, trimethoprim and sulphamethoxazole readily enter host cells resulting in a higher intracellular concentration to kill *L. monocytogenes* (Slutsker and Schuchat, 1999).

**Antibiotic Resistance**

The ability of bacteria to grow in the presence of antibiotics that would normally inhibit their growth or kill them is termed antibiotic resistance. The vast majority of bacteria are susceptible to antibiotics and these bacteria out compete the resistant strains which are in the minority. When antibiotics are administered, they kill off the susceptible majority, thus allowing the resistant minority to begin to thrive. These resistant bacteria
will start to dominate the environment until once again they are out-competed by the susceptible population. Continuous exposure to antibiotics over time causes the population of sensitive bacteria to decrease while the resistant bacteria increase.

The increasing number of bacteria developing antibiotic resistance is of growing global concern. Particularly worrisome is the emergence of resistance to multiple antibiotics that complicate treatment options, and in some cases, results in an untreatable infection (McEwen and Fedorka-Cray, 2002). There are two main reasons for the antibiotic resistance scare. One is the overuse and misuse of antibiotics prescribed by physicians, and the other is the widespread use of antibiotics in agriculture (Charpentier and Courvalin, 1999). Many physicians feel pressured by parents of sick children to administer antibiotics even if not appropriate. Instead of allowing the child’s sick body to fight off the infection over a longer period of time, physicians prescribe antibiotics to cure the infection in an abbreviated time frame. Thus, the overuse of antibiotics, especially penicillins, has resulted in bacteria becoming resistant to those medications. Animals raised for meat and milk consumption are often given antibiotics in their food and water to both treat and prevent infectious diseases and to promote their growth (McEwen and Fedorka-Cray, 2002). However, many of these antibiotics are also used to treat humans with a bacterial infection. According to McEwen and Fedorka-Cray (2002), antibiotic resistance has emerged in zoonotic enteropathogens, like Salmonella and Campylobacter, commensal bacteria, Escherichia coli and Enterococci, and bacterial pathogens of animals such as Pasteurella spp. and Actinobacillus spp. Thus, the carcasses of agricultural animals and their products are contaminated with antibiotic resistant strains of pathogenic bacteria that can be transmitted to humans via the food
chain (McEwen and Fedorka-Cray, 2002; Anonymous, 2005b). This transmission can complicate treating foodborne infections because the bacteria may not be susceptible to the same human clinical antibiotics that are also used with agriculture.

Bacteria utilize three mechanisms to become resistant to antibiotics: natural resistance, single point mutations, and new gene acquisitions (Salyers and Whitt, 2002). Natural, or innate resistance, is due to the phenotypic characteristics of the bacterium. The outer membrane of Gram-negative bacteria provides a great shield to two antibiotics, rifampin and vancomycin (Salyers and Whitt, 2002). Rifampin, which inhibits RNA synthesis, is too hydrophobic to enter through the membrane porins and the vancomycin molecule is too large to enter into the same porins. Mycobacterium spp. have mycolic acids on their cell surface. Mycolic acids give it a “waxy” surface that makes it difficult for antibiotics to enter the cell. Leuconostoc spp., Pediococcus spp., and some Enterococcus spp. may have different amino acid pairs on their pentapeptides. For example, instead of D-ala-D-ala, they may add another amino acid pair like D-ala-D-ser. This could cause innate resistance to antibiotics that bind to penicillin binding proteins like vancomycin and cephalosporins.

Single point mutations are a second vehicle to gain resistance to antibiotics. This is an acquired resistance. Single point mutations, or genetic alterations, may occur in many places of the prokaryotic cell. One example is a porin mutation, which results in the reduced uptake of antibiotics into the cell. A decrease in the pore size or channel size blocks the entry of antibiotics with large molecular structures. Porin mutations can result in multiple antibiotic resistances such as those to β-lactams, quinolones, and chloramphenicol (Salyers and Whitt, 2002). Another example of single point mutations
is alteration of the target site of the antibiotic. Mutation of the penicillin binding proteins (transglycolases and transpeptidases) changes the binding specificity of $\beta$-lactams like methicillin, penicillin, and ampicillin. This mutation often occurs with Gram-positive bacteria and causes problems clinically (Salyers and Whitt, 2002). This single point mutation causes the encoding of a new protein, penicillin binding protein2', that replaces normal transpeptidase and allows peptidoglycan cross-linking to occur even in the presence of a $\beta$-lactam.

Mutation of the 30S subunit of the bacterial ribosome can lead to resistance to aminoglycosides (e.g., gentamicin, streptomycin, and kanamycin) (Rusin and Gerba, 2001). Mutation of the gyrase $\beta$-subunit in DNA prevents the binding of quinolones (e.g., ciprofloxacin, enrofloxacin, naladixic acid) causing resistance to these drugs (Rusin and Gerba, 2001). Rifampin resistance occurs by the mutation of RNA polymerase $\beta$-subunit to prevent rifampin binding. A resistance to trimethoprim and sulfamethoxazole occurs with mutations to dihydrofolate reductase and dihydropteroate synthetase enzymes, respectively (Salyers and Whitt, 2002). These point mutations cause a decrease in the affinity of these enzymes for this antibiotic duo in the tetrahydrofolate pathway.

The third mechanism, and another form of acquired resistance, is the acquisition of new genes that confer antibiotic resistance. These newly acquired genes that carry antibiotic resistances are transferred from foreign DNA, such as plasmids, transposons, or phages, and incorporated into the bacterium’s chromosomal or plasmid DNA (Salyers and Whitt, 2002). The acquisition of antibiotic resistant genes from foreign bacteria can lead to modifying the antibiotic target, enzyme inactivation of the antibiotic, adding functional groups to chemically change the antibiotic and active efflux pumps.
One way new gene acquisition causes antibiotic resistance is by modification of the antibiotic’s target. For example, new acquisitions of *ermA, erm B, ermC*, and/or *ermD* genes encode for the synthesis of an enzyme called rRNA methylase. This enzyme methylates adenine on the 23S rRNA of the 50S ribosome subunit. This modification prevents the binding of three classes of antibiotics; streptogramins, macrolides, and lincosamides (Salyers and Whitt, 2002). Another example of modification of the antibiotic’s target is the acquisition of *vanA* and *vanH* gene products that confer resistance to vancomycin. These two gene products change the D-ala-D-ala pentapeptide into D-ala-D-dehydroxybutyrate. This mutant pentapeptide is not recognized by vancomycin, which recognizes D-ala-D-ala, but is still used by transpeptidases and transglycolases to promote peptidoglycan growth (Salyers and Whitt, 2002). Biavasco et al., (1996) found that enterococci could spread *vanA* to *L. monocytogenes* by conjugation in vitro for vancomycin resistance.

A second way that acquisition of new genes confers antibiotic resistance is by encoding for enzymes that inactivate the antibiotic (Rusin and Gerba, 2001). β-lactamases are enzymes that cleave β-lactam rings and render them inactive. Gram-positive bacteria secrete β-lactamases in the extracellular fluid while Gram-negative bacteria secrete β-lactamases into the periplasmic space (Salyers and Whitt, 2002). β-lactamases are a diverse group of enzymes that work only with a specific β-lactam. β-lactamases, depending on specificity, inactivate various penicillins, cephalosporins, carbapenems, and monobactums. It is possible to counteract β-lactamases by mixing a β-lactam with a β-lactamase inhibitor, like clavulanic acid or sulbactam. These inhibitors have no effect on the bacteria but prevent the activity of β-lactamases so that the
antibiotic can kill or cease the growth of the bacteria. Some bacteria have already
evolved to be resistant to β-lactamase inhibitors, but in general these inhibitors have
renewed the use of some old and obsolete β-lactams, such as ampicillin (Salyers and
Whitt, 2002).

The addition of chemical functional groups to an antibiotic is another way the
acquisition of new genes causes resistances to antibiotics. This keeps the antibiotic from
actively fitting to its target. The addition of phosphorylases, adenylases, or acetylases to
aminoglycosides can inactivate them. Another example is the enzyme chloramphenicol
acetyltransferase (CAT) which transfers an acetyl group to chloramphenicol to inactivate
it (Rusin and Gerba, 2001). The addition of these new functional groups changes the
physical property of the antibiotic, such as its size, and changes the chemical properties,
such as charge and hydrophobicity. Thus, the antibiotic is no longer capable of binding
specifically to its appropriate site on the cell membrane and it’s entry into the cell is
inhibited or reduced.

Active efflux is another example of new gene acquisitions that cause antibiotic
resistance (Rusin and Gerba, 2001; Godreuil et al., 2003). Antimicrobial efflux pumps
aid to pump antimicrobials out of the bacterial cell. Antibiotics that inhibit protein
synthesis, like tetracyclines and macrolides, must first gain entry into the cell and then
accumulate in high enough concentrations to bind to the ribosome. Bacteria use protein
efflux pumps to remove antimicrobials from the cytoplasm as fast as they enter.
Therefore, the antibiotic cannot accumulate in high enough concentrations to bind to the
ribosome and stop protein synthesis. A common efflux pump is one that rids the cell of
tetracycline, which is used in agricultural animals (Rusin and Gerba, 2001). The genes
tetA – tetG in Gram-negative bacteria and tetK, tetL in Gram-positives encode for the protein efflux pump (Salyers and Whitt, 2002). After its synthesis, the efflux pump is inserted into the cell membrane and it pumps out tetracycline before its intracellular concentration gets high enough to be effective. Staphylococcus spp. has two unique efflux pumps. An ATP-dependent transport protein pump that effluxes specific macrolides and a cytoplasmic membrane pump that effluxes quaternary ammonium compounds (QACs) (Salyers and Whitt, 2002). QACs are antimicrobials frequently used to disinfect contaminated environments such as hospitals and food processing plants. Moreover, genes for QAC efflux pump have been found on plasmid and chromosomal DNA (Salyers and Whitt, 2002). Thus, it may be possible that L. monocytogenes could acquire QAC efflux genes from Staphylococcus spp. in a biofilm at a poultry processing plant. Lemaitre et al., (1998) suggested that the emergence of multi-drug resistant L. monocytogenes, resistant to benzalkonium chloride, hexamidine diisethionate, and ethidium bromide, could be due to the acquisition of a replicon that originated in a Staphylococcus spp. The authors also reported that Listeria spp. from poultry carcasses were more multi-drug resistant than those Listeria isolated from humans, red meats, cheeses, wild-birds, and dairies.

A quick, easy, and safe way for bacteria to acquire resistances to antibiotics is through resistant gene transfers from other bacteria in their community. The transformation of chromosomal genes is an intraspecies transfer (Salyers and Whitt, 2002). This occurs naturally as the bacteria divides and grows, during bacterial cell lyses or from bacteriophages.
Chromosomal DNA escapes into the environment and this free DNA can be picked up by a bacterium of the same species and incorporated into their DNA.

The most common mode of gaining antibiotic resistance genes is by conjugation, which is an inter-species transfer. Conjugation, or bacterial sex, is a direct cell-to-cell transfer of DNA by a conjugative pili or direct membrane attachment. Plasmids and conjugative transposons are two types of conjugative elements. Plasmids are mobile extrachromosomal DNA segments that carry among other things virulence factors and antibiotic resistance genes (Salyers and Whitt, 2002). One cause for the increase in multi-drug resistances is that plasmids can acquire multiple resistance genes from other bacteria. Thus, a bacteria acquiring one plasmid could allow for resistances to many antibiotics and an increase in virulence. Flamm et al. (1984) found that the broad host range antibiotic resistance plasmid pAMβ1 was transferred by conjugation from *Streptococcus faecalis* to 9 out of 15 *L. monocytogenes* strains. That study also noted that the *L. monocytogenes* strains could transfer the plasmid to other *L. monocytogenes* strains or back to *S. faecalis* without antibiotic pressure.

A plasmid can gain more than one resistance gene if they are carried on transposons (Salyers and Whitt, 2002). Transposons, or mobile elements, are DNA segments that can insert themselves into another bacterium’s chromosomal or plasmid DNA without homologous recombination. The one or more resistance genes are located between two insertion points.

Conjugative transposons are normally located in the bacterial chromosome but can transfer themselves from the chromosome of the donor to the chromosome of the
recipient or integrate themselves into their plasmid. The conjugative transposons, or CTn, free themselves from the donor chromosome and form a closed circle. CTn is then transferred to the receiving cell by conjugation where it joins its DNA. Salyers and Whitt (2002) stated that conjugative transposons could transfer as many antibiotic resistance genes as do plasmids especially with Gram-positive bacteria like L. monocytogenes.

**Antibiotic Resistance with Listeria monocytogenes**

Antibiotic resistance is low in L. monocytogenes relative to other Gram-positives such as Enterococcus spp., Staphylococcus spp., and Streptococcus spp. (Heger et al., 1997; Troxler et al., 2000; Mayrhofer et al., 2004; Walsh et al., 2001). Heger et al. (1997) noted in a study that all 66 clinical strains of L. monocytogenes were sensitive to ampicillin, gentamicin, trimethoprim/sulphamethoxazole, ampicillin-sulbactam, and meropenem and noted that in vitro susceptibility has not changed during the last decades. Charpentier et al. (1995) noted that 1,100 isolates of Listeria spp. from cases of listeriosis, food, and the environment were all sensitive to the primary drugs of choice, ampicillin and gentamicin. Troxler et al. (2000) tested clinical, mammalian, and environmental isolates and noted that listeriae are naturally sensitive or intermediate to tetracyclines, aminoglycosides, penicillins, macrolides, and chloramphenicol. However, Troxler et al. (2002) and Heger et al. (1997) noted that cephalosporins have no effect in vitro and that this innate resistance is due to the lack of appropriate penicillin binding proteins in the Listeria cytoplasmic membrane.
These studies agree with the findings of Hansen et al. (2005) who studied the antimicrobial resistance profiles of human listeriosis isolates from 1958 – 2001 in Denmark. Although these authors found very few resistances to ciprofloxacin (1 isolate) and gentamicin (2 isolates), the rest of the 106 samples were sensitive. The authors found no difference in the susceptibility patterns between serotypes 1/4 and 4. Overall, they concluded that the antibiotic resistance of *L. monocytogenes* has not increased in Denmark since 1958 (Hansen et al., 2005).

*L. monocytogenes* isolated from food seem to follow the same pattern as those from clinical and environmental isolates. Mayrhofer et al. (2004) tested 304 *L. monocytogenes* samples from beef, pork, and poultry. The authors observed no isolate resistant to tetracycline, penicillin, gentamicin, vancomycin, cotrimoxazol, erythromycin, chloramphenicol, or streptomycin. Walsh et al. (2001) noted that no multiple resistances occurred with *L. monocytogenes* from retail store samples but did have one isolate resistant to gentamicin. Walsh’s study suggests that the overall incidence of antibiotic resistance is low in *Listeria* spp. with tetracycline resistance being the most frequently observed. Barbuti et al. (1992) examined antibiotic resistance profiles of *L. monocytogenes* in Italian meat products. Most were sensitive to the antimicrobials, and the authors observed no plasmids from the recovered bacteria in the sausage products. Thus, at the present time, it seems unlikely to acquire a multi-drug resistant listeriosis infection from contaminated foods (Slade and Collins-Thompson, 1990, and Navratilova et al., 2004).

However, there are reasons to be concerned that *L. monocytogenes* may become resistant to antibiotics like other Gram-positive bacteria have. Since *L. monocytogenes* is
ubiquitous and also found in the gastrointestinal tracts of broilers and in food processing plants, there is an abundance of antibiotic resistant plasmids available to receive during conjugation from *Enterococcus* spp. and *Staphylococcus* spp. (Charpentier and Courvalin, 1999; Navratilova et al., 2004). This could explain why human isolates of *L. monocytogenes* exhibit a strong resistance to tetracycline (Charpentier and Courvalin, 1999). Tetracycline resistance is increasing and is likely due to the use of this antibiotic in human therapy and as an agricultural animal feed supplement (Charpentier and Courvalin, 1999). A more dangerous antibiotic resistance gene acquisition from conjugation has been found causing resistance to trimethroprim (Charpentier et al. 1995). The acquisition of the *dfrD* gene by *L. monocytogenes* is thought to have originated from *Staphylococcus* spp. and could be transferred while in the gut microflora (Charpentier and Courvalin, 1997).

Biavasco demonstrated a dangerous scenario (1996). In this study, the *vanA* gene responsible for vancomycin resistance was transferred from *Enterococcus faecium* to *L. monocytogenes*. The recipient *L. monocytogenes* had an amazing MIC ≥ 256 µg/ml to vancomycin, the last drug used for multi-drug resistant Gram-positive infections. The study also proved that *L. monocytogenes* could transfer this gene to other *Listeria* spp. It is possible for *L. monocytogenes* to obtain vancomycin resistance and/or other resistant genes from *Enterococcus* spp. or other *Listeria* spp. A species-related ability to acquire and donate multiple resistance genes has been shown with *Listeria* spp. (Walsh et al., 2001). In that study, *Listeria innocua* was reported to have a high number of antibiotic resistances and multi-drug resistances compared to other *Listeria* spp.
Besides tetracycline, *L. monocytogenes* has also shown resistance to fluoroquinolones like ciprofloxacin and norfloxacin (Godreuil et al., 2003; Navratilova et al., 2004). Five clinical *L. monocytogenes* isolates were shown to be resistant to both ciprofloxacin and norfloxacin while neither was clonally related in a study by Godreuil et al. (2003). The resistance was due to efflux of the drug from the cell by proteins after the expression of the *Listeria* drug efflux (*Lde*) gene. This gene also conferred resistance to ethidium bromide and acridine orange. This study showed that even though fluoroquinolones are not an option for listeriosis treatment, when this class of drug is used for other infections, or even in agricultural animal use, it can select for ciprofloxacin resistant *L. monocytogenes*.

*L. monocytogenes* is fully capable of evolving into a multi drug resistant pathogen (Charpentier and Courvalin, 1999) like other poultry associated bacteria. The first multi-drug resistant strain was found in France in 1988 from an 84 year old patient with meningoencephalitis (Poyart-Salmeron et al., 1990). Walsh et al. (2001) noted that while resistance to antibiotics is presently low in *L. monocytogenes*, the range of antibiotics to which resistances has been acquired is wide. Resistances of concern are to ampicillin, penicillins, tetracycline, and gentamicin. Soriano et al. (1995) found an increase in the MIC values for *L. monocytogenes* to ampicillin and suggested that this resistance could be more widespread than previously thought.

Although rare, the emergence of multi-drug resistant *L. monocytogenes* has been observed in meat products. Facinelli et al. (1991) isolated *L. monocytogenes* from a frankfurter involved in a case of listeriosis and found it to have resistances to streptomycin, sulphamethoxazole, kanamycin, erythromycin, gentamicin, and rifampin.
Multi-drug resistant *L. monocytogenes* has also been isolated from both raw and cooked chicken (Yucel et al., 2005). These authors noted that 66% of the isolated strains were resistant to ampicillin and trimethoprim / sulphamethoxazole. The first case of high antibiotic resistant *L. monocytogenes* with food in Spain was reported by Rota et al. (1996). These authors found that 80% of the isolated strains from pork sausage had resistance to 9 antibiotics. Resistance to drugs that are used in the clinical treatment of human listeriosis were also encountered in that study. One isolate from that study showed resistance to all 12 drugs tested. From these studies it is clear that the emergence of multi-drug resistant strains of *L. monocytogenes* is possible with further processed foods. These results are contrary to the conclusions drawn by Slade and Collins-Thompson, (1990) and Navratilova et al., (2004).

It is thought that eventually *L. monocytogenes* will evolve into a multi-drug resistant pathogen like other Gram-positives (Charpentier and Courvalin, 1999). Espaze and Reynaud (1988) correctly predicted the emergence of resistant strains of *Listeria* spp. as food production techniques changed and the numbers of *Listeria* spp. in food processing plants increased, thus increasing the opportunity for the transfer of antibiotic resistance genes. The emergence of multi-drug resistant *L. monocytogenes* could be similar to that pattern of multi-drug resistant *Salmonella* and *Campylobacter* spp. from the overuse of antibiotics employed during poultry production (Rota et al., 1996; Yucel et al., 2005).
**Listeria monocytogenes and Integrated Poultry Production**

Since *L. monocytogenes* is an ubiquitous pathogen, it is likely to end up in poultry production (Yucel et al., 2005). The organism can contaminate the feed and water that is given to poultry flocks (Husu et al., 1990; Ojeniyi et al., 1996). *L. monocytogenes* has been recovered from domestic chickens and turkeys (Gray and Killinger, 1966). After consumption of contaminated feed or water, birds can shed the pathogen via feces. Listeria in birds is usually non pathogenic and does not cause production problems or losses in the broiler-grow out phase (Bailey et al., 1989). However, low level contamination of broilers, who are carriers, could lead to the persistent contamination of poultry slaughter and further processing plants and eventually to the contamination of RTE poultry products.

*L. monocytogenes* has been observed in low numbers in poultry breeder flocks and in the hatchery. Ojeniyi et al. (1996) isolated *L. monocytogenes* in 4.7% of cecal samples from parent flocks that provide broilers; three out of the five parent flocks sampled were positive for *L. monocytogenes*. Cox et al. (1997) recovered *L. monocytogenes* on just 6% of egg shell fragments and only 1% on chick transport pads. Cox et al. (1997) concluded that the hatchery was not a critical entry point for *L. monocytogenes* in poultry production. Eggs would seem to supply adequate growth conditions for this pathogen but the occurrence of lysozyme may reduce its presence more than those of Gram-negative pathogens like *Salmonella* and *Campylobacter* spp. (Lou and Yousef, 1999).

Unlike *Salmonella* and *Campylobacter* spp., the grow out phase of broiler production does not seem to be a likely reservoir for *L. monocytogenes* either. Husu et al.
Husu et al. (1990) and Bailey et al. (1990) looked at the colonization of *L. monocytogenes* in orally inoculated chicks. Husu et al. (1990) and Bailey et al. (1990) found that *L. monocytogenes* colonized the ceca like *Salmonella* and *Campylobacter* spp., but unlike these other two pathogens, *L. monocytogenes* was completely shed out of the body through the feces in most chicks within 9 days after inoculation.

Husu et al. (1990) followed the path that *L. monocytogenes* takes within the body of chicks. Five days after the inoculation, they detected *L. monocytogenes* in the livers of all the birds. Also, the pathogen was detected inside the surface of epithelium cells and inside macrophages of the gastrointestinal surface 5 – 28 days post inoculation. The numbers of *L. monocytogenes* recovered from the chicks decreased dramatically in 2 weeks from the liver and the digestive tract due to a good immune response. The authors also noted that no pathological changes occurred in chicks that survived. However, 18% of chicks given a very high dose (10⁸) died within two days. *L. monocytogenes* was isolated from the liver, gastrointestinal tract, and from muscle tissue. In these dead chicks, liver lesions and hepatic parenchymal cell death was observed. The authors also noted that, in surviving chicks, no *L. monocytogenes* was present in the muscle tissue. Long term *L. monocytogenes* colonization of the ceca was only observed in a single chick 28 days after inoculation at a level of 2 x 10⁴ CFU. Therefore, only a very small percentage of broilers could be considered carriers of *L. monocytogenes*, and broiler production, as a whole, is unlikely to be an important reservoir.

Bailey et al. (1990) found that younger chicks (inoculated at day 1 of age) were more susceptible to *L. monocytogenes* colonization than older birds (inoculated at day 14 and 35 of age). The authors also noted that an inoculation dose of 10⁶ caused more
colonization that a dose of $10^2$. Bailey et al. (1990), like Husu et al. (1990), recovered *L. monocytogenes* from the liver and ceca.

Fecal shedding of *L. monocytogenes* can, however, cause a significant problem when it comes to poultry processing (Husu et al., 1990). Even though only a small percentage of birds (0 – 1.3%) may carry *L. monocytogenes* into a poultry slaughter plant (Cox et al., 1997), *L. monocytogenes* contamination increases significantly in the later stages of poultry processing, especially after immersion chilling (Cox et al., 1997; Ojeniyi et al., 1996; Reiter et al., 2005). Cox et al. (1997) noted that 40% of samples in their study were positive for *L. monocytogenes* post-chill compared to 0 – 1.3% pre-scald.

Ojeniyi et al. (1996) studied the presence of *L. monocytogenes* throughout a Danish poultry processing plant and the products produced. They found 0.3 – 18.7% of the samples were positive for *L. monocytogenes* from different abattoirs and 62 different clones were discovered. Since the same strain of *L. monocytogenes* was found first on feces from a transfer crate and then later found in the slaughter plant environment, Ojeniyi and the other scientists believe that live broilers can introduce the pathogen into a slaughter plant. That study also showed that *L. monocytogenes* colonized most of the processing line and equipment which could lead to the contamination of the carcass and products. Environmental samples showed that the processing line, scald tank, defeathering machine, evisceration machine, chiller, floors and walls were all positive for *L. monocytogenes*. The same study also showed that cooked poultry and marinated poultry were also positive. The authors noted that some of the same *L. monocytogenes* strains found on the scalder, picker, and evisceration machines were also present on cooked poultry.
Reiter et al. (2005) compared the prevalence of *Campylobacter* spp. and *L. monocytogenes* on fresh and frozen chicken from a Brazilian processing plant. This study revealed that *L. monocytogenes* was present on 35.6% of environmental, water and poultry samples as compared to 16.6% for *Campylobacter* spp. positive samples. *Campylobacter* spp. was more prevalent in the gastrointestinal tract and gall bladder and *L. monocytogenes* was more prevalent in the liver and on the skin of carcasses. In fact, *L. monocytogenes* was found in higher numbers on the fresh and frozen parts than was *Campylobacter* spp. The authors noted that *Campylobacter* was more prevalent than *L. monocytogenes* before evisceration but that after evisceration and chilling *L. monocytogenes* was more prevalent. This high prevalence of *L. monocytogenes* was seen with both the fresh and frozen chicken parts. The incidence of positive *L. monocytogenes* were frozen breasts, 100%; frozen wings, 93%; fresh breasts, 83.3%; fresh wings, 80%; frozen legs, 60%; and fresh legs, 50%. Miettinen et al. (2001) reported similar results from retail store chicken parts: legs were 68%, drumsticks 67%, breasts 52%, and wings 50% positive for *L. monocytogenes*.

Contaminated processing equipment is a major reason why *L. monocytogenes* presence in a poultry slaughter plant increases dramatically from very low on live broilers arriving at the plant to 23 – 62% positive carcasses at the retail level (Bailey et al., 1989; Miettinen et al., 2001; Hudson and Mead, 1989). The small percentage of broilers carrying *L. monocytogenes* into both the scalding and chiller water may further contaminate a large number of carcasses following down the processing line (Johnson et al., 1990). This would explain the high percentage of positive chicken parts in studies by Objeniyi et al. (1996), Miettinen et al. (2001) and Reiter et al. (2005). Hudson and Mead
(1989) sampled a poultry slaughter plant on three visits and found the automatic carcass opener positive on all trips. This machine gains entry into the visceral cavity and if *L. monocytogenes* is present in the feces, it could spread the pathogen to every other bird that came in contact with that machine afterwards.

Berrang et al. (2000) noted that changing the evisceration equipment may have a dramatic effect on decreasing the presence of *L. monocytogenes* in a slaughter plant. This study surveyed three slaughter plants. All three plants showed low *L. monocytogenes* prevalence pre-chill, and in two of the plants, the prevalence was still low post-chill. However, in one plant post-chill carcasses were 22% positive for *L. monocytogenes*. This plant then underwent major renovations and installed new evisceration equipment. Upon its completion, the researchers could not recover any *L. monocytogenes* from post-chill carcass samples.

Employee handling of carcasses also has a major impact on the high numbers of *L. monocytogenes* after carcass chilling (Genigeorgis et al., 1989). They reported that 34.7% of employees handling carcasses were positive for *L. monocytogenes* and those employees cutting legs and wings had the highest presence on their gloves. When employee hands and gloves were sampled at 3 distinct areas post-chill, the following percents were positive for *L. monocytogenes*: rehanging chilled carcasses, 20%; cutting carcasses, 45.5%; and packaging parts, 59%. Thus, the more employees handle a carcass, or its parts, tends to increase the presence of *L. monocytogenes* (Lawrence and Gilmour, 1994).
Although the hatchery and broiler grow-out environments are not reservoirs for \( L.\) monocytogenes, it appears that \( L.\) monocytogenes is present in poultry processing plants (Cox et al. 1997). Processing plants can harbor the same serogroups of virulent \( L.\) monocytogenes like 1/2b, 1/2c (Bailey et al., 1989), 1/2a and 4b (Miettinen et al., 2001) that have been associated with human listeriosis outbreaks. Equipment design and sanitation, as well as employee hygiene, play major roles in the eventual contamination of a carcass with \( L.\) monocytogenes. Changes in operational sanitation procedures from SSOPs, good manufacturing practices and an established HACCP plan should be used to decrease the incidence of contamination (Berrang et al., 2000). It is clear that this reservoir is not from the acute levels of \( L.\) monocytogenes on live broilers or their feces, but from the processing environment and machines that eventually contaminate carcasses (Ojeniyi, 1996; Cox et al., 1997).

\( L.\) monocytogenes in raw poultry product has been directly linked to the contamination of a poultry further processing plant and then eventually to the contamination of a cooked poultry product after persisting within the plant (Berrang et al., 2002 and 2005). Berrang et al. (2002 and 2005) sampled from the environment, raw product and cooked product in a poultry further processing plant. This cook plant had established precautionary measures for \( L.\) monocytogenes contamination. The raw and cook sides were physically separated by walls and each room had its own positive air pressure. Employees wore different color uniforms that were permitted in only one room of the plant. Mandatory foot baths and hand wash stations were also used. However, in the 2002 study, 15 different ribogroups of \( L.\) monocytogenes were isolated. The floor drains were the site of most environmental \( L.\) monocytogenes residents. As many as 5
different subtypes were discovered in a single drain on the same sampling day. Seven of 9 pooled samples of raw product, coming into the facility from a raw poultry processing plant, were also positive for *L. monocytogenes*. The same subtypes of *L. monocytogenes* found on the incoming raw product were also isolated from both the raw and cook side environments and even found on the same sampling day (2005). Four subtypes became persistent throughout the plant. Three of those resident strains were found on raw poultry entering the plant and then reisolated throughout the plant for an extended period of time. Berrang et al. (2005) found one subtype entered the further processing plant on the raw poultry, established residence, and was later detected on the cook side environment where it apparently contaminated fully cooked poultry product.

Persistent strains of *L. monocytogenes* may establish dominance and residence in poultry processing plants for years, and different processing plants have different strains that persist (Miettinen et al., 2001; Lunden et al., 2003). The ability of a strain to establish a dominant residency is controlled by genetics (Lemaitre et al., 1998; Autio et al., 2003). Lemaitre et al. (1998) found that *L. monocytogenes* recovered from poultry carcasses were 47% more resistant to sanitizers like benzalkonium chloride, hexamidine diisethionate, and ethidium bromide than were strains recovered from dairy foods, humans, wild birds, and the environment. Extrachromosomal DNA originating from *Staphylococcus* was found in all the resistant *L. monocytogenes* strains. Thus, sensitive populations of *Listeria* could acquire these genes and become persistent within the processing plant. Cellular attachment and the production of biofilms also have a major effect on whether a strain can survive and become persistent within a processing plant environment (Lawrence and Gilmour, 1994).
Mafu et al. (1990) studied the attachment of *L. monocytogenes* on stainless steel, polypropylene, glass, and rubber surfaces at both ambient (20°C) and cold (4°C) temperatures. Scanning electron micrographs indicated that *L. monocytogenes* could attach to both the rough porous surfaces (rubber and propylene) and the smooth non-porous surfaces (glass and stainless steel). At 20°C, *L. monocytogenes* was able to attach to all these surfaces without the aid of visible fibrils and extracellular secretions. After 1 hour, an extracellular polysaccharide secretion (EPS) was seen surrounding the attached cells. At 4°C, after 1 hour, EPS were only observed on the glass and polypropylene surfaces. This study illustrated that *L. monocytogenes* can quickly attach to all of these surfaces that are widely used in processing plants and form biofilms which protect them from sanitizers, allowing them to be persistent within a plant without adequate sanitation.

Attachment by persistent strains to processing equipment that comes in direct contact with the product may lead to the cross contamination of the final product. Lunden et al. (2003) found that food processing equipment, like slicers, spiral freezers, packing machines and conveyors, contaminated with consistent *L. monocytogenes* pulse field gel electrophoresis (PFGE) profiles can lead to the contamination of the final product by these same subtypes. Thus, persistent virulent strains of *L. monocytogenes* that are attached to equipment contacting the food product increase the chances for cooked product contamination and possible outbreaks of listeriosis. This was also the case with broiler carcass contamination in a poultry slaughter plant. The complexity of processing machines has added to the problem due to inadequate sanitation in hard to reach areas (Lunden et al., 2003). This is especially a problem with those machines having close metal-to-metal parts and gaskets (J. F. Frank, University of Georgia, Athens,
GA, personal communication). A better understanding of the behavior of persistent and non-persistent strains is vital for planning *L. monocytogenes* prevention strategies in a poultry processing plant (Lunden et al., 2003).

Although *L. monocytogenes* is not seen as a problem with raw poultry processing, loss of control for this organism in a poultry slaughter plant can lead to the contamination of a poultry further processing plant. If the strain can persist under favorable conditions without the proper sanitation of equipment that is in contact with food, then contamination of a cooked poultry product is likely. *L. monocytogenes* can then grow very well on refrigerated sliced chicken and turkey (Glass and Doyle, 1989). These researchers inoculated sliced chicken and turkey with $10^2$ CFU/g of *L. monocytogenes* and stored the poultry at 4.4°C for up to 6 weeks. In 2 weeks, the sliced poultry had *L. monocytogenes* levels of $10^6$ cfu/g and in 4 weeks the products were visibly spoiled and contained $10^8$ cfu/g. Genigorgis et al. (1989) also observed increased *L. monocytogenes* presence on raw chicken parts after 4 days storage at 4°C. Packaged livers, drumsticks, and wings were sampled after processing and were, respectively, 33.3%, 36.7%, and 70% positive. After 4 days storage at 4°C, the percentage of positive pieces increased to 40%, 52%, and 72% positive, respectively.

**Ultraviolet Irradiation**

Ultraviolet (UV) light has a long history as an antimicrobial treatment and is gaining acceptance among the food industry as an effective bactericide. Typical applications of UV light for disinfection occur with sewage treatments, water treatment, and in hospital rooms. Food and poultry processors are interested in UV irradiation
because it is a non-thermal process that can effectively eliminate food borne pathogens, such as *L. monocytogenes*, while maintaining the food’s fresh organoleptic or sensory qualities (Wallner-Pendelton et al., 1994).

UV light occurs between visible light and X-rays on the electromagnetic spectrum. The range of UV light is from 100 – 400 nm. UV-A (315 – 400 nm) is long wave UV and is responsible for the tanning of human skin. UV-B (280 – 315 nm) can cause sunburns that may eventually lead to skin cancer. UV-C (200 – 280 nm), or short wave, is called the germicidal range because it has bactericidal properties and the vacuum UV (100 – 200 nm) range can be absorbed by most substances but can only be transported in a vacuum (Bolton, 1999). For most purposes, UV light is generated by the passing of an electrical discharge through low-pressure mercury vapor that is enclosed in glass tubes or bulbs which are referred to as germicidal lamps (Rusin and Gerba, 2001).

The USDA-FSIS defines irradiation as the process of exposing food to radiant energy in order to reduce or eliminate bacteria, making it safer and more resistant to spoilage. UV irradiation of poultry and other meats does not produce radiant energy as a byproduct, because the energy used is not powerful enough to make the product “radioactive”. According to FSIS, during irradiation safety testing, scientists used much higher levels of radiation than what is approved for poultry and found no cancerous or toxic effects in animals that consumed the irradiated poultry (Anonymous, 1999b).

The federal government has experimented with irradiated foods since after World War II as a means to produce safe food for soldiers in the field. The Food and Drug Administration (FDA), which must approve irradiation as a food additive, first approved it for fruits, vegetables, and spices in 1963 for the elimination of insects and
microorganisms. To prevent trichinosis, the FDA approved irradiation of pork in 1985. In May 1990, FDA ruled that poultry meat irradiated at the absorbed dose of 3 kGy is safe for human consumption while effective at controlling foodborne illness (Anonymous, 1999b). By 1992, FSIS had approved the guidelines for raw packaged poultry meat to be irradiated. Finally, in 1997, FDA determined that irradiation of raw meat was safe for consumption. The FSIS final rule stated that irradiated poultry must not exceed 3 kGy, the package must be air permeable, and that all irradiated products must bare the radura label (Anonymous, 1999b).

UV irradiation of poultry products has many advantages to both the food processor and the consumer. Besides the destruction of *L. monocytogenes* and *Salmonella* spp., UV irradiation has no negative effects on sensory qualities like color and rancidity on broiler breast meat. UV light systems can be easily installed into a facility at low costs and even operated under cold conditions (Stermer et al., 1987). Thus, UV energy can kill targeted pathogens while the product is held at low temperatures for packaging or other further processing. Since little to no heat is given off by the UV lamps (Rowan et al., 1999), the consumer gets a fresh raw product that is microbiologically safer with a longer shelf life.

UV-C light absorbed by the bacteria damages nucleotides (Wright et al., 2000) and to a lesser degree denatures cell wall proteins (Bachmann, 1975). DNA is damaged as a result of photochemical changes of the pyrimidine bases. A cross-linking between adjacent thymine and cytosine (pyrimidine nucleoside bases) in the same DNA strand produces cyclobutyl pyrimidine dimers and the amount of cross-linking that occurs is directly proportional to the amount of UV-C light absorbed by the bacterial cell.
(Guerrero-Beltran and Barbosa-Canovas, 2004). These lethal dimers are scattered throughout the bacterial population during UV irradiation (Lado and Yousef, 2002). As a result of the production of the cyclobutyl pyrimidine dimers, the DNA helix becomes distorted and DNA transcription and replication are blocked (Guerrero-Beltran and Barbosa-Canovas, 2004; Lado and Yousef, 2002). The denaturation of membrane proteins can inhibit ionic flow into or out of the bacterial cell and also depolarizes the cell membrane. These can influence metabolism of the bacterium. Therefore, the application of 254 nm of shortwave UV energy on raw poultry can be an effective listeriocide.

UV energy has shown to be extremely effective at destroying surface bacteria on smooth surfaces like agar plates and stainless steel due to an increase of direct UV-C light absorption by the bacterium. Sumner et al. (1996) noted an almost complete elimination (99.9%) of *Salmonella typhimurium* on BHI agar plates. As a result of UV irradiation, the authors observed a 7 log decrease in *Salmonella typhimurium* compared to the control plates. Another study by Yousef and Marth (1988) yielded similar results with *L. monocytogenes* on Tryptose agar. The authors described a decrease of 7 orders of magnitude of *L. monocytogenes* in only 4 minutes at a dose of 100 µW/cm². During the first minute of UV irradiation, *L. monocytogenes* became inactivated. During the second minute, the maximum rate of *L. monocytogenes* inactivation occurred and during the fourth minute, death diminished and *L. monocytogenes* viability began to level off. Kim et al. (2002) looked at the UV irradiation of stainless steel chips to decrease populations of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes*. At a UV dose of 500 µW/cm² for 3 minutes, the authors noted that *E. coli* O157:H7 was totally destroyed (6.19 log decrease). *S. Typhimurium*, and *L. monocytogenes* were more UV resistant but were
still significantly reduced ($P \leq 0.05$) by 4.9 and 4.1 logs respectively. Stermer et al. (1987) examined the effects of UV irradiation on populations of *Pseudomonas*, *Micrococcus*, and *Staphylococcus* spp. on Tryptic soy agar plates. The authors observed a 99.9% kill. These studies show that on smooth surfaces UV-C light is an excellent surface disinfectant.

Scientists have noted significant decreases in foodborne pathogens on the surface of meats as well, but in general UV is not as effective as reported for smoother surfaces. Stermer et al. (1987) noted this when they looked at beef plate meat (smooth meat surface) and beef round (rough meat surface) samples. UV irradiation of the beef plate meat was highly effective with a $>97\%$ kill of *Pseudomonas*, *Micrococcus*, and *Staphylococcus* spp. while beef round samples had a much lower kill effect. The authors believed that the rough surface of beef round was due to cutting across muscle fibers. The bacteria inoculated on the surface were then able to enter the meat and avoid direct surface UV exposure. The smooth beef plate meat, on the other hand, had meat fibers that were parallel to its surface, allowing bacteria to remain on the surface. The authors concluded that since UV irradiation does not penetrate opaque matter, the bacteria under the surface is shielded or protected from UV light on the meat’s surface. Kim et al., (2002) noticed also that UV irradiation was less effective at reducing bacteria on chicken meat, with or without skin, than on a smoother surface (stainless steel) at the same dose. *Salmonella typhimurium* and *L. monocytogenes* were not significantly reduced ($P > 0.05$) on the chicken meat as they were on the stainless steel chips. The authors concluded that the feather follicles on the skin surface were hiding places for the bacteria and they were sheltered from the UV exposure.
However, other scientists have shown UV irradiation of poultry meat and eggs to be an effective bactericide. Wallner-Pendelton et al., (1994) observed a 61% decrease (0.5 log reduction) of *S.* Typhimurium on UV treated (dose of 82,560 to 86,400 µW/cm²) broiler carcass halves as compared to untreated halves. Sumner et al., (1996) noticed an effective 80.5% reduction of *S.* Typhimurium on inoculated poultry skin. Berrang et al., (1995) reported that UV light at 254 nm significantly (P < 0.05) reduced the incidence of *S.* Typhimurium on egg shells that were inoculated by drop suspension. Results for eggs with a fecal smear inoculate were not as dramatic. This was due to the shell not being clean and smooth. The researchers showed that 5 minutes at 1,000 µW/cm² was an effective bactericidal dose. An important conclusion from this study was that the UV irradiation treatment had no effect on the hatchability of the fertile broiler eggs. These studies offer hope for the UV irradiation of poultry products. While complete destruction was not observed as with smoother surfaces, the significant reduction of *S.* Typhimurium suggests that UV irradiation could also be effective for controlling *L.* monocytogenes on processed poultry.

As effective as a continuous dose of UV irradiation is on inhibiting bacteria, pulsed UV power may be even more effective. Pulsed UV power requires many mega watts of peak electrical power that is dissipated into a light source in a very short time, for example 1 µs (MacGregor et al., 1998). According to these authors, pulsed power creates a greater electrical field than that of continuous UV. As a result, it produces more shortwave UV light. They tested it by inoculating *E. coli* O157:H7 and *L. monocytogenes* on tryptone soya yeast extract agar. The results showed a 99.9% decrease with *E. coli* O157:H7 and a 99% decrease with *L. monocytogenes* for only 64 pulses at a
1 μs duration. The author then noted that the greater number of light pulses resulted in a greater log order decrease. This resulted in a 6 log decrease for *E. coli* O157:H7 and a 7 log decrease for *L. monocytogenes* when 512 pulses at 1 μs were performed. Rowan et al. (1999) found similar results with 200 pulses of high intensity UV on pathogens grown on tyrpene soy yeast extract agar. That study revealed a greater than four log decrease in populations of *Bacillus cereus* and *L. monocytogenes*; a 4 – 5 log decrease in *Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella enteritidis*; and a 5 – 6 log decrease in *E. coli*. These two experiments show that pulsed UV, at least on agar, is greater at decreasing pathogens than continuous UV exposure.

As with other antimicrobials, some bacteria are able to survive after being injured by UV irradiation. UV resistance depends on many factors such as the genetic makeup of the bacteria, humidity, and cell wall structure. Dry *L. monocytogenes* cells are more UV resistant than moist *L. monocytogenes* cells (Yousef and Marth, 1988). Due to the teichoic acids embedded in the peptidoglycan layer, Gram-positive bacteria are more resistant to UV damage than Gram-negative species (Lado and Yousef, 2002). Chang et al. (1985) discovered that *Bacillus subtilis* spores were 9 times more UV resistant than vegetative cells.

Sublethally UV injured cells often undergo mutations and express cellular repair systems in order to survive (Lado and Yousef, 2002). These UV tolerant repair systems, according to Rusin and Gerba (2001) are due to sunlight exposure. One such system is the SOS system for cellular repair in response to cellular damage. The SOS system’s first action is to activate RecA protease. RecA protease is involved with recombination DNA repair due to the formation of pyrimidine dimers. RecA also cleaves the repressor protein
coded for by the *lexA* gene (Rusin and Gerba, 2001). LexA is a stable protein coded by the *lexA* gene and functions to repress operons that have genes coding for repair functions. The SOS system is prone to error and can result in mutations if an incorrect base is inserted into the DNA (Rusin and Gerba, 2001).

Another repair system is the UVR system. The UVR system is involved in two repair systems, short-patch (20 nucleotides) and long-patch (1500 nucleotides) repair (Rusin and Gerba, 2001). This system has 3 genes; *uvrA*, *uvrB*, and *uvrC* that encode for an endonuclease to recognize thymine dimers or other distortions. These endonucleases make incisions on the 5’ end of the damaged site and another incision on the 3’ end of the DNA strand. Repair synthesis of the UV damaged DNA is then performed by DNA polymerases (Rusin and Gerba, 2001).

Photoreactivation is another repair system mechanism used by bacteria that is enhanced by visible blue light (Sastry et al., 2000). Photoreactivation occurs when photolyase monomerizes the pyrimidine dimers of the split nucleic acids due to UV exposure (Stevens et al., 1998). If sublethally UV injured cells are exposed to light between 330 – 480 nm, then photoreactivation may increase the number of viable microorganisms present. To avoid this regrowth, UV irradiated food products should be packaged and maintained in a dark package (Guerrero-Beltran and Barbosa-Canovas, 2004). Photoreactive cells have greater UV tolerance than cells that are non-photoreactive. Photoreactivated cells can be more UV resistant when exposed to UV light for a second time (Sastry et al., 2000; Hoyer, 1998).

Plasmid DNA also plays a major role in UV light tolerance and resistance. The production of colicin I by some bacteria seem to make them 3 – 4 times more UV
resistant than noncolicin producing bacteria strains of *E. coli* and *Salmonella typhimurium* (Howarth, 1966). Plasmids carrying antibiotic resistance genes are also capable of carrying UV resistant R-factors. For example, R46, a conjugative plasmid in *Salmonella typhimurium*, confers resistance to ampicillin, sulfonamides, streptomycin, and tetracycline and also decreases the killing effect of UV light while increasing mutagenic effects (Rusin and Gerba, 2001). R46 carries the UVP (Ultra Violet Protection) gene that enables UV tolerance or resistance and has an increased capacity for repair errors leading to mutations (Mortelmans and Stocker, 1979). Like antibiotic resistant genes, R-factors can be transferred to a new cell by conjugation (Rusin and Gerba, 2001). R2 is a plasmid in *Pseudomonas aeruginosa* that encodes for a true UV inducible mutagenic DNA repair system that can be transferred to other pathogens via conjugation (Simonson et al., 1990).

Since the overuse of antibiotics in both human and animal medicine has lead to the emergence of antibiotic resistant pathogens, it is not unreasonable to think that the overuse of UV irradiation of poultry meat could lead to the emergence of more UV resistant pathogens. UV surviving pathogens on poultry meat could be capable of spreading both antibiotic and UV resistant genes to other bacteria. Staley et al. (1988) performed experiments to determine if UV irradiation of sewage effluent selected for antibiotic resistant coliforms and fecal coliforms. Two doses of UV were evaluated; a high intensity UV (1.11 x 10^5 µW-sec/cm^2) and a low intensity dose (1.82 x 10^4 µW-sec/cm^2). Overall, in coliforms there was no selection of antibiotic resistance in either the low or high UV dose. However, in fecal coliforms there was a slight increase in antibiotic resistance with the high UV dose. A 2–4 % increase in tetracycline and
streptomycin was observed. Meckes (1982) studied coliforms that were passed through sludge treatment in wastewater effluents for antibiotic resistance before and after UV treatment. While the dose of 45,000$\mu$W-sec/cm$^2$ effectively killed the coliforms, antibiotic resistance to tetracycline and chloramphenicol increased significantly from 3.1% and 0.2% to 16.6% and 1.3% respectively. The author believed that R-factors played a role in the increased tetracycline resistance by preventing the drug from entering the cell wall. Meckes also noted that the percentage of antibiotic resistance transfer to a donor increased from 43% to 49% after the UV treatment. Another dangerous scenario in which UV irradiation might increase antibiotic resistance is that if UV lyses the cell of a lysogenic bacterial strain then bacteriophages would be released which could transduce antibiotic resistance genes to susceptible pathogens (Krcmery, 1999).


CHAPTER 3

EFFECT OF GERMICIDAL ULTRAVIOLET LIGHT ON THE ANTIBIOTIC RESISTANCE PROFILES OF *LISTERIA MONOCYTOGENES*\(^1\)

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Abstract

Listeria monocytogenes isolates (161) recovered from a poultry further processing plant were tested using the microdilution method for susceptibility to 19 antibiotics. Minimal inhibitory concentrations (MICs) were determined and the isolates were labeled as either “sensitive” or “intermediate / resistant” to the drugs. In vitro testing of susceptibility showed all isolates were susceptible to all antibiotics except for ceftriaxone (156 isolates intermediate / resistant), oxacillin with 2% NaCl (146 isolates intermediate / resistant), ciprofloxacin (60 isolates intermediate / resistant), clindamycin (40 isolates intermediate / resistant), and tetracycline (5 isolates intermediate / resistant). Four strains were chosen from these 161 isolates based on differing subtypes and antibiotic resistance profiles for ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline. These 4 different strains were inoculated onto raw skinless chicken breasts and subjected to ultraviolet (UV) irradiation (λ = 254 nm) at a dose of 1,000 µW/cm² for 5 min. The UV treatment resulted in no significant changes in the antibiotic resistance profiles for any of the 4 strains compared to non UV irradiated controls. This data suggests that UV irradiation of raw poultry breasts could be applied as an antimicrobial food process without selecting for increased resistances to ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline.

Keywords: antibiotic resistance, ultraviolet irradiation, Listeria monocytogenes, poultry processing, food safety.
Introduction

*Listeria monocytogenes* is an important foodborne pathogen responsible for the two deadliest foodborne disease outbreaks in the history of the United States (Anonymous 1999; Ryser 1999). Foodborne listeriosis, caused by *L. monocytogenes*, can have a fatality rate between 20 – 30% (Slutsker and Schuchat 1999) and is especially dangerous for immunocompromised individuals. Antibiotic treatment may be indicated for susceptible individuals; the primary drugs of choice are ampicillin or ampicillin in combination with an aminoglycoside such as streptomycin or gentamicin (Charpentier and Courvalin 1999). Patients allergic to penicillins can be treated with trimethoprim and sulphamethoxazole in tandem to treat listeriosis (Poyart-Salmeron et al. 1990).

Antibiotic resistance is less common in *L. monocytogenes* than other Gram-positive bacteria (Heger et al. 1997; Troxler et al. 2000; Walsh et al. 2001; Mayrhofer et al. 2004). Nevertheless, *L. monocytogenes* has been shown to be capable of evolving into a multi-drug resistant pathogen (Charpentier and Courvalin 1999). Since *L. monocytogenes* can be found in the gastrointestinal tracts of animals and in food processing plants, it can be exposed to conjugation with *Enterococcus* spp. and *Staphylococcus* spp. carrying plasmids coding for antibiotic resistance (Charpentier and Courvalin 1999; Navratilova et al. 2004). This route has been shown to occur for trimethoprim (Charpentier et al. 1995) and vancomycin (Biavasco 1996) resistance. *L. monocytogenes* has already begun to show resistance to antibiotics used in both human infections and in agricultural animal use.
Strains resistant to tetracycline (Charpentier and Courvalin 1999) and ciprofloxacin (Godreuil et al. 2003; Navratilova et al. 2004) have been reported. Antibiotic resistant *L. monocytogenes* have been recovered from meat (Rota et al. 1996) and poultry products (Yucel et al. 2005).

Ultraviolet light at a wavelength of 200 – 300 nm (UV) is an effective antimicrobial processing technique for water (Rusin and Gerba 2001) and on the surface of fresh meat and poultry (Stermer et al. 1987; Wallner-Pendleton et al. 1994). UV light absorbed by bacteria damages nucleotides (Wright et al. 2000) and cell wall proteins (Bachmann 1975). UV treatment causes the formation of pyrimidine dimers which distorts the DNA double helix blocking replication and transcription (Lado and Yousef 2002; Guerrero-Beltran and Barbosa-Canovas 2004).

Contaminated raw poultry meat can be a vector for *L. monocytogenes* entry into a further processing plant (Berrang et al. 2002 and 2005). In a 2005 study by Berrang et al., the same subtypes of *L. monocytogenes* isolated on raw poultry shipped to a further processing plant from a slaughter plant were also recovered from both the raw and cook sides of the further processing plant on the same sampling day. This study also showed that one particular subtype isolated on raw poultry became persistent throughout the plant, even on the cook side, where it contaminated a cooked poultry product. UV irradiation could be used as an antimicrobial food process immediately prior to packaging at a slaughter plant to reduce the presence of *L. monocytogenes* on raw poultry shipped to a further processing plant.
Although UV irradiation can be an effective bactericide, it can also cause mutation which may result in antibiotic resistance among the surviving bacteria. There is some evidence for this in the literature (Meckes 1982; Staley et al. 1988), but it is not known how \textit{L. monocytogenes} would be affected. The objective of this study was to determine if UV treatment applied to raw chicken meat to kill \textit{L. monocytogenes} prior to arrival at a further processing plant results in any changes in the antibiotic resistance profiles of surviving \textit{L. monocytogenes}.

\textbf{Materials and Methods}

\textit{LISTERIA MONOCYTOGENES CULTURES}

In an earlier study by Berrang et al. (2005), 161 \textit{L. monocytogenes} isolates were recovered from a poultry further processing plant in Georgia. All isolates were subtyped by a gene sequencing method. All isolates were originally frozen on beads (Microbank™, Pro-Lab Diagnostics, Austin, TX, 78754) and stored at -80°C. One bead for each isolate was placed in 5 ml Brain-Heart Infusion (BHI) broth (Criterion, Hardy Diagnostics, Santa Maria, CA) tube and incubated for 24 h at 35°C. Ten \(\mu\)l from each BHI broth tube was struck for isolation on a Modified Oxford (MOX) agar plate (Oxford Medium Base plus Modified Antimicrobial Supplement; Oxoid) and incubated for 24 h at 35°C. An isolated esculin-positive colony (small grey colony with black halo) was chosen for antibiotic susceptibility testing and grown on a BHI slant (Criterion, Hardy Diagnostics, Santa Maria, CA) for 24 h at 35°C. Slants were stored at 4°C until testing. Four strains were chosen based on differing subtype and antibiotic resistance profiles to 4 specific antibiotics (ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline)
as determined by the microdilution method described by Trek Diagnostic Systems, Inc.
for Gram-positive isolates (Cleveland, Ohio) (Table 1). Susceptibility breakpoints were established by Clinical and Laboratory Standards Institute (CLSI) (CLSI, Wayne, PA) for Gram-positive organisms. Cultures of the 4 subtypes were maintained on BHI agar slants at 4°C for inoculation of the raw, skinless, boneless chicken breasts.

ANTIBIOTIC AGENTS

Antibiotics (19) for susceptibility tests were selected considering (a) antibiotics used in the clinical treatment of listeriosis (ampicillin-AMP; gentamicin-GEN; penicillin-PEN; streptomycin-STR; trimethoprim / sulphamethoxazole-SXT), (b) antibiotics used to treat various human diseases (erythromycin-ERY; vancomycin-VAN; clindamycin -CLI; clarithromycin-CLA; ciprofloxacin-CIP; linezolid-LZD; gatifloxacin- GAT; levofloxacin-LEVO; rifampin-RIF; cefazolin-FAZ), (c) antibiotics used in animal production (quinupristin/dalfopristin-SYN; tetracycline-TET) and (d) antibiotics that L. monocytogenes has previously shown to be intermediate / resistant to (oxacillin with 2% NaCl-OXA; ceftriaxone-AXO). These 19 antibiotics cover a wide range of drug classes including macrolides, aminoglycosides, cephalosporins, β-lactams, fluoroquinolones, antimetabolites, a glycopeptide, a streptogramin, a lincosamide, a tetracycline, an ansamycin, and an oxazolidinone. A Sensititre® GPN2F panel (Trek Diagnostic Systems, Inc., Cleveland, OH), with the 19 antibiotics at various doubling dilutions, was used for susceptibility testing by the microdilution method according to CLSI standards for L. monocytogenes (CLSI, Wayne, PA).
MICRODILUTION PROCEDURE

The equipment and supplies needed for the microdilution method were from Trek Diagnostic Systems Inc. (Cleveland, Ohio). Three control strains; *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* 29212, were tested for GPN2F panel effectiveness once a week during the test period. Isolates maintained on BHI slants were first streaked for isolation on MOX and incubated for 24 h at 35°C. An isolated colony was then used to make a lawn on TSA with 5% sheep blood agar (BAP, Remel, Lexmark, Kansas) incubated for 24 h at 35°C. Culture from the BAP was added to five ml tube of sterile demineralized water (Sensititre®, Trek Diagnostic Systems, Inc., Cleveland, OH) to a turbidity equal to a 0.5 McFarland standard. Ten µl of the inoculated water was then transferred to an 11 ml tube of Mueller Hinton broth with TES buffer and 2-2.5% lysed horse blood (Sensititre®, Trek Diagnostic Systems, Inc., Cleveland, OH), 50 µl was then dispensed into each well of the GPN2F panel. Panels were incubated for 18 h at 35°C and the turbidity in each well was read manually using the SensiTouch® machine (Sensititre®, Trek Diagnostic Systems, Inc., Cleveland, OH). Results were recorded in the SWIN® computer program (Sensititre®, Trek Diagnostic Systems, Inc., Cleveland, OH). All MIC breakpoints were established by CLSI for either *L. monocytogenes* or for other Gram-positive organisms for which *L. monocytogenes* has no established MICs for that drug (Table 2). Due to a one well (dilution) variance with the microdilution method (P. Fedorka-Cray, USDA-ARS, Athens, GA, personal communication), isolates were classified as “sensitive” or “intermediate/resistant”.

75
UV EQUIPMENT

A shortwave UV bench lamp (Model XX-15S, Ultra-Violet Products, Inc., Upland, CA) with two bulbs generating light at a wavelength of 254 nm, 115 V, 60 Hz, and 0.68 Amps was used in this experiment. The bench lamp was placed on an exposure stand (Model XX-15 Series, Ultra-Violet Products, Inc. Upland, CA) with adjustable shelves for different levels of exposure. The entire unit was placed in a hood with a UV impenetrable clear glass sliding door. UV intensity was measured in microwatts per centimeter squared (\(\mu W/cm^2\)) using an intensity meter (Model J-225, Blak-Ray®, Ultra-Violet Products, Inc., Upland, CA). The shelves were adjusted so that each sample’s inoculated surface received a UV-C intensity of 1,000\(\mu W/cm^2\). UV-C intensity (1,000\(\mu W/cm^2\)) at the expected location for the breast’s surface was measured before and after each trial for accuracy and repeatability.

UV TREATMENT PROCEDURE

Fresh, raw boneless and skinless chicken breasts were purchased from an Athens, GA supermarket and transported on ice to the laboratory within 30 min. The study was conducted over a 12 week period, 3 weeks for each of the 4 strains. Every week, 20 breasts were purchased and breasts with any visible defects (cuts or bruises) were discarded. Ten were inoculated for UV treatment group; another 10 were inoculated for the non UV treatment control group. Five trials were performed each week. Each trial consisted of 2 UV treated breasts and 2 breasts for the control group (5 trials x 2 breasts for UV treatment x 2 breasts for control = 20 breasts). All four breasts for each trial were placed on sterile wypalls (Kimberly-Clark Corporation, Roswell, GA) for inoculation. The day prior to inoculation, a sterile inoculation needle was used to pick growth off of a
BHI slant (stored for maintenance at 4°C) containing the desired *L. monocytogenes* subtype (J,N,L, or X) for that week, placed in a 5 ml BHI broth tube, and incubated at 35°C for 24 h. The inoculum, after 24 h incubation, contained around 1.0 x 10^9 colony-forming units (CFU) per ml. One serial dilution of the original inoculum was then prepared in a 9 ml PBS dilution blank. Ten µl from the dilution blank was placed on the center of each breast so that each breast received 10^6 CFU/ml. The dilution blank with the inoculum remained on ice throughout the entire procedure and was later used to confirm the number of the inoculum load. The inoculum was spread evenly across the surface of each breast with a sterile plastic bioloop. The breasts were held at room temperature (~25°C) on the counter for 5 min to allow the inoculum to dry. After 5 min, 2 of the 4 breasts were chosen at random and put on the UV bench tray for UV treatment. The tray was adjusted under the lamps so that the breasts would receive 1,000 µW/cm^2^ and the UV lamps were turned on for 5 min. Concurrently, untreated control breasts stayed on the counter for 5 min. After exposure, the UV lamps were turned off and all treated and control breasts were aseptically placed into individual Ziploc® bags for storage (24 h at 4°C). This was done to simulate shipping of the raw poultry from the slaughter plant to a poultry further processing plant. The next day, 50 ml sterile PBS was placed into each Ziploc® bag and the bag was vigorously shaken by hand for 60 s. Serial dilutions of the rinse were prepared in PBS and plated onto duplicate MOX plates for *L. monocytogenes* recovery. MOX plates were incubated for 24 hours at 35°C; 3 typical *L. monocytogenes* colonies from each sample were struck to BHI slants (incubated for 24 h at 35°C) for maintenance (stored at 4°C) and antibiotic resistance testing as described above.
STATISTICAL ANALYSIS

A chi-square test for independence was performed to determine if any significant changes in the antibiotic resistance profiles to ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline occurred post UV treatment. P values less than 0.05 were considered significant.

Results and Discussion

Excepting AXO, OXA, CIP, and CLI, all antibiotics used in this study inhibited the growth of *L. monocytogenes* (Table 3). MICs for OXA were in the resistant range for 146 of the 161 isolates (91%). MIC values for AXO amounted to intermediate / resistant in 156 isolates (97%). MICs for CIP were in the intermediate / resistant range for 60 isolates (37%). Forty isolates (25%) had MICs in the intermediate range for CLI. Five isolates (3%) were resistant to TET. The 4 strains (J, N, L, and X) chosen for this study were used because they showed resistance patterns common to other *L. monocytogenes* strains isolated from food processing plants and meats. According to Heger et al (1997) and Troxler et al (2002), newer generations of cephalosporins, like AXO, have no in-vitro effect on *L. monocytogenes*. This innate resistance is due to the lack of specific penicillin binding proteins 3 and 5 in the *Listeria* cytoplasmic membrane (Heger et al. 1997).

Troxler et al. (2002) also noted that *L. monocytogenes* were naturally resistant to OXA. TET and CIP acquired resistances have also been observed from *L. monocytogenes* isolated from foods (Walsh et al. 2001; Navratilova et al. 2004). The acquired resistance to CIP could be due to the acquisition of the *Listeria* drug efflux (*Lde*) gene that would pump the antibiotic out of the cell before its concentration is high enough to be
bactericidal (Godreuil et al. 2003). The acquired resistances to TET and CIP may be due to the use of these antibiotics in broiler production which could select for *L. monocytogenes* strains resistant to these antibiotics (Charpentier and Courvalin 1999; Godreuil et al. 2003). Therefore, the 4 strains used in our study are comparable to the types of strains recovered from a poultry further processing plant environment and a poultry RTE product.

UV treatment, at a dose of 1,000µW/cm² for 5 min, had no significant effect (P values > .05) on the antibiotic resistance profiles of any of the 4 *L. monocytogenes* subtypes tested (Table 4). Table 4 shows the percentage of intermediate / resistant *L. monocytogenes* isolates for the UV treatment group and the control group. Strains J and N both exhibited no changes in their resistance profiles between the UV treated and control groups to the 4 antibiotics. Strain L for the UV treatment group was noted to have small non significant changes in both AXO and OXA (drugs not used to treat listeriosis in humans) compared to the control group. UV treated isolates of subtype X, the strain with the most antibiotic resistances, had a small non significant change in resistance to OXA and a 20% decrease (P = 0.07) in resistance to CIP in the UV treatment group compared to the control group.

Our data is different than what has been reported in wastewater and sewage effluent studies. Although no significant changes in the antibiotic resistance profiles occurred due to UV treatment in this study, Meckes (1982) did find that UV irradiation of wastewater effluents did significantly select for coliforms with higher resistances to TET (13%) and chloramphenicol (1%). That study also showed that the percentage of
antibiotic resistance transfer to a donor increased from 43 – 49% after UV irradiation. Another study by Staley et al. (1988) also showed that fecal coliforms increased resistances to TET and STR by 2 – 4% after UV irradiation.

In conclusion, UV irradiation had no significant effect on the antibiotic resistance profiles of *L. monocytogenes* and no increase in resistances to these drugs occurred under the conditions of this study. These data suggest that UV irradiation of raw poultry breast fillets could be applied as an antimicrobial treatment without selecting for an increase in resistances to AXO, OXA, CIP, and TET among the surviving population. More research is needed to determine if UV irradiation selects for increased resistances to the drugs used to treat human listeriosis like ampicillin, gentamicin, streptomycin, penicillin, and trimethoprim / sulphanethoxazole
References


Table 3.1. Antibiotic resistance profiles of *Listeria monocytogenes* isolates prior to exposure to UV irradiation\(^1\).

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Ceftriaxone</th>
<th>Oxacillin(^2)</th>
<th>Ciprofloxacin</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>16 / (\geq 32)</td>
<td>I/R (\geq 4)</td>
<td>I/R (\leq 1)</td>
<td>S (\leq 4)</td>
</tr>
<tr>
<td>N</td>
<td>16 / (\geq 32)</td>
<td>I/R (\geq 4)</td>
<td>I/R (2 / \geq 4)</td>
<td>I/R (\leq 4)</td>
</tr>
<tr>
<td>L</td>
<td>16 / (\geq 32)</td>
<td>I/R (\leq 2)</td>
<td>S (\leq 1)</td>
<td>S (8 / \geq 16)</td>
</tr>
<tr>
<td>X</td>
<td>16 / (\geq 32)</td>
<td>I/R (\geq 4)</td>
<td>I/R (2 / \geq 4)</td>
<td>I/R (8 / \geq 16)</td>
</tr>
</tbody>
</table>

\(^{\ast}\)S = Sensitive, I/R = Intermediate / Resistant.

\(^1\)UV irradiation was administered at a dose of 1,000 \(\mu\)W/cm\(^2\) for 5 min.

\(^2\)with 2% NaCl

\(^3\)MIC (Minimum Inhibitory Concentration) values determined by the microdilution method for *L. monocytogenes*, Clinical and Laboratory Standards Institute (CLSI, Wayne, PA).

\(^4\)CLSI established breakpoints for *Staphylococcus* spp.

\(^5\)Veterinarian established breakpoints for *Staphylococcus aureus*.

\(^6\)CLSI established breakpoints for *Enterococcus* spp.

\(^7\)Veterinarian established breakpoints for *Enterococcus* spp.
Table 3.2. Antibiotics and extent of dilutions in GPN2F* panels for *Listeria monocytogenes* including breakpoint criteria in terms of minimum inhibitory concentration (MIC)

<table>
<thead>
<tr>
<th>Antibiotic Agent</th>
<th>Dilution Range (µg/ml)</th>
<th>Susceptible (µg/ml)</th>
<th>Intermediate / Resistant (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin¹,²</td>
<td>0.12 – 16</td>
<td>≤ 2</td>
<td>-</td>
</tr>
<tr>
<td>Cefazolin¹,³</td>
<td>2 – 16</td>
<td>≤ 8</td>
<td>16 / ≥ 32</td>
</tr>
<tr>
<td>Ceftriaxone¹,⁴</td>
<td>8 - 64</td>
<td>≤ 8</td>
<td>16 / ≥ 32</td>
</tr>
<tr>
<td>Ciprofloxacin¹,⁵</td>
<td>0.5 – 2</td>
<td>≤ 1</td>
<td>2 / ≥ 4</td>
</tr>
<tr>
<td>Clarithromycin¹,⁴</td>
<td>1 - 8</td>
<td>≤ 2</td>
<td>4 / ≥ 8</td>
</tr>
<tr>
<td>Clindamycin¹,⁶</td>
<td>0.5 – 4</td>
<td>≤ 0.5</td>
<td>1 - 2 / ≥ 4</td>
</tr>
<tr>
<td>Erythromycin¹,⁷</td>
<td>0.25 – 8</td>
<td>≤ 0.5</td>
<td>1 - 4 / ≥ 8</td>
</tr>
<tr>
<td>Gatifloxacin¹,⁵</td>
<td>1 – 8</td>
<td>≤ 2</td>
<td>4 / ≥ 8</td>
</tr>
<tr>
<td>Gentamicin¹,³</td>
<td>2 – 16; 500</td>
<td>≤ 4</td>
<td>8 / ≥ 16</td>
</tr>
<tr>
<td>Levofoxacin¹,⁵</td>
<td>0.25 – 8</td>
<td>≤ 2</td>
<td>4 / ≥ 8</td>
</tr>
<tr>
<td>Linezolid¹,⁵</td>
<td>0.5 – 8</td>
<td>≤ 2</td>
<td>4 / ≥ 8</td>
</tr>
<tr>
<td>Oxacillin with 2% NaCl¹,⁸</td>
<td>0.25 – 8</td>
<td>≤ 2</td>
<td>≥</td>
</tr>
<tr>
<td>Penicillin¹,²</td>
<td>0.06 – 8</td>
<td>≤ 2</td>
<td>-</td>
</tr>
<tr>
<td>Quinupristin / Dalfopristin¹,⁵</td>
<td>0.12 – 4</td>
<td>≤ 1</td>
<td>2 / ≥ 4</td>
</tr>
<tr>
<td>Rifampin¹,⁷</td>
<td>0.5 – 4</td>
<td>≤ 1</td>
<td>2 / ≥ 4</td>
</tr>
<tr>
<td>Streptomycin¹,⁵</td>
<td>1000</td>
<td>≤ 512</td>
<td>≥ 1024</td>
</tr>
<tr>
<td>Tetracycline¹,⁷</td>
<td>2 – 16</td>
<td>≤ 4</td>
<td>8 / ≥ 16</td>
</tr>
<tr>
<td>Drug</td>
<td>MIC Range</td>
<td>Breakpoint</td>
<td>Breakpoint</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Trimeth / Sulphameth&lt;sup&gt;1,9&lt;/sup&gt;</td>
<td>1 / 19 – 4 / 76</td>
<td>≤ 0.5 / 9.5</td>
<td>1 / 19 – 2 / 38 / ≥ 4 / 76</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;1,7&lt;/sup&gt;</td>
<td>1 - 32</td>
<td>≤ 4</td>
<td>8 – 16 / ≥ 32</td>
</tr>
</tbody>
</table>

<sup>*Trek Diagnostic Systems, Inc. (Cleveland, OH)</sup>

<sup>1</sup>Breakpoint MICs established by CLSI (2004)

<sup>2</sup>CLSI established breakpoints for *Listeria monocytogenes*

<sup>3</sup>Veterinarian established for CLSI

<sup>4</sup>CLSI established for *Staphylococcus* spp.

<sup>5</sup>CLSI established for *Enterococcus* spp.

<sup>6</sup>Veterinarian established for *Staphylococcus* spp.

<sup>7</sup>Veterinarian established for *Enterococcus* spp.

<sup>8</sup>Veterinarian established for *Staphylococcus aureus*

<sup>9</sup>Veterinarian established for *Streptococcus pneumoniae*
Table 3.3. Number and percentage intermediate / resistant<sup>1</sup> isolates from 161 *Listeria monocytogenes* isolates recovered from a poultry further processing plant.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number Isolates I / R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin&lt;sup&gt;2,5&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefazolin&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ceftriaxone&lt;sup&gt;2,5&lt;/sup&gt;</td>
<td>156 (97%)</td>
</tr>
<tr>
<td>Ciprofloxacin&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>60 (37%)</td>
</tr>
<tr>
<td>Clarithromycin&lt;sup&gt;2,5&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Clindamycin&lt;sup&gt;2,7&lt;/sup&gt;</td>
<td>40 (25%)</td>
</tr>
<tr>
<td>Erythromycin&lt;sup&gt;2,8&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Gatifloxacin&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Gentamicin&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Linezolid&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Oxacillin with 2% NaCl&lt;sup&gt;2,9&lt;/sup&gt;</td>
<td>146 (91%)</td>
</tr>
<tr>
<td>Penicillin&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Quinupristin / Dalfopristin&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Rifampin&lt;sup&gt;2,8&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Streptomycin&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Tetracycline&lt;sup&gt;2,8&lt;/sup&gt;</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Trimethoprim / Sulphamethoxazole&lt;sup&gt;2,10&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;2,8&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
From Table 3.3

1MIC (Minimum Inhibitory Concentration) values determined by the microdilution method for *L. monocytogenes*, Clinical and Laboratory Standards Institute (CLSI, Wayne, PA).

2Breakpoint MICs established by CLSI (2004)

3CLSI established for *L. monocytogenes*

4Veterinarian established for CLSI

5CLSI established for *Staphylococcus* spp.

6CLSI established for *Enterococcus* spp.

7Veterinarian established for *Staphylococcus* spp.

8Veterinarian established for *Enterococcus* spp.

9Veterinarian established for *Staphylococcus aureus*

10Veterinarian established for *Streptococcus pneumoniae*
Table 3.4. Number and percentage of intermediate / resistant<sup>1</sup> *Listeria monocytogenes* isolates<sup>2</sup> for control groups and UV<sup>3</sup> treatment groups.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Treatment</th>
<th>Strain</th>
<th>( J )</th>
<th>( N )</th>
<th>( L )</th>
<th>( X )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>Control</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td>28/30 (93%)</td>
<td>30/30 (100%)</td>
<td></td>
</tr>
<tr>
<td>Oxacillin&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Control</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td>0/30 (0%)</td>
<td>9/30 (30%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td>1/30 (3%)</td>
<td>10/30 (33%)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Control</td>
<td>0/30 (0%)</td>
<td>30/30 (100%)</td>
<td>0/30 (0%)</td>
<td>26/30 (87%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>0/30 (0%)</td>
<td>30/30 (100%)</td>
<td>0/30 (0%)</td>
<td>20/30 (67%)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Control</td>
<td>0/30 (0%)</td>
<td>0/30 (0%)</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>0/30 (0%)</td>
<td>0/30 (0%)</td>
<td>30/30 (100%)</td>
<td>30/30 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>MIC (Minimum Inhibitory Concentration) values determined by the microdilution method for *L. monocytogenes*, Clinical and Laboratory Standards Institute (CLSI, Wayne, PA).

<sup>2</sup>30 isolates per treatment group for each 4 subtypes.

<sup>3</sup>UV irradiation was administered at a dose of 1,000 µW/cm<sup>2</sup> for 5 min.

<sup>4</sup>with 2% NaCl
CHAPTER 4

EFFECTS OF ULTRAVIOLET TREATMENT OF RAW CHICKEN FILLETS ON THE RECOVERY OF *LISTERIA MONOCYTOGENES* AND MEAT COLOR

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1 S. A. Lyon, D. L. Fletcher, and M. E. Berrang, to be submitted to Poultry Science
**Abstract**

Raw chicken breast fillets were subjected to ultraviolet (UV) irradiation (dose of 1,000 µW/cm² for 5 min at a wavelength of 254 nm) to evaluate its potential to reduce *Listeria monocytogenes* on raw product before shipment to a poultry further-processing plant. Boneless, skinless breast fillets were inoculated with 4 different strains of *L. monocytogenes* 5 min prior to treatment. After the UV treatment, breast fillets were stored at 4°C for 24 h. Enumeration of remaining *L. monocytogenes* was performed using the spread plate method on Modified Oxford Medium agar. An approximate 2 log reduction in viable *L. monocytogenes* was observed with all 4 strains on UV irradiated breast fillets as compared to the non-irradiated breast fillets. The UV treatment had no effects on meat color (lightness, redness, and yellowness) on day of treatment or after 7 d storage. This study suggests that UV irradiation of raw breast fillets at a slaughter plant can significantly reduce *L. monocytogenes* without negatively affecting meat color. This process could be used to reduce the negative impact of raw poultry as a transmission vector of *L. monocytogenes* into a poultry further-processing plant.

*Key words: L. monocytogenes, ultraviolet irradiation, poultry processing, meat color*
Introduction

Listeria monocytogenes is an important foodborne pathogen of particular concern to immunocompromised individuals. Susceptible individuals have underlying health conditions with decreased T-cell mediated immunity (Mackaness, 1962). Listerialis often has serious sequelae diseases such as septicemia, meningitis, and endocarditis (Donnelly, 2001) that can result in a fatality rate between 20 – 30% (Slutsker and Schuchat, 1999). Pregnant women are also susceptible to foodborne listeriosis, and the fetus is most often stillborn or spontaneously aborted (Martin and Fisher, 1999). Two of America’s deadliest outbreaks of foodborne disease were a result of listeriosis (Ryser, 1999; Anonymous, 1999a). In 2002, fully cooked poultry products were involved in a multi-state listeriosis outbreak (Anonymous, 2002). This outbreak, in the northeast United States, resulted in 46 confirmed cases, 7 deaths and 3 stillbirths or abortions. The responsible company voluntarily recalled 27.4 million pounds of processed chicken and turkey meat, the largest meat recall ever in the United States.

Due to its ubiquitous nature, L. monocytogenes readily ends up in integrated poultry production (Yucel et al., 2005). The slaughter plant environment can become a persistent reservoir of this pathogen (Cox et al., 1997). Cox et al. (1997) noted that the percentage of positive L. monocytogenes broiler samples increased from 1.3% (pre-scald) to 40% (post-chill) as the carcasses moved through the raw processing lines. Contaminated raw poultry can be a vector for L. monocytogenes entry into a poultry further-processing plant (Berrang et al., 2002 and 2005). L. monocytogenes has the ability to survive under unfavorable environmental and processing conditions (Fenlon, 1999) making this pathogen difficult to control in a poultry further-processing plant.
Strains persisting in the poultry further-processing plant environment, especially on food contact surfaces, are capable of contaminating cooked poultry meat before packaging (Berrang et al., 2005). In a 2005 study by Berrang et al., the same subtypes of *L. monocytogenes* found on raw product coming in to a poultry further-processing plant were also isolated from both the raw and cook sides of the plant on the same sampling day. This dispersion of *L. monocytogenes* was apparent despite physical wall barriers and positive air pressure on each side of the plant. This same study also showed that one particular subtype isolated from raw poultry meat became persistent throughout the plant, even on the cook side, where it contaminated a cooked poultry product.

Ultraviolet (UV) light between 200 – 300 nm has been reported to have germicidal properties on the surface of fresh meats including fresh poultry (Stermer et al., 1987; Wallner-Pendleton et al., 1994). UV light absorbed by the bacteria primarily damages nucleotides (Wright et al., 2000) and to a lesser extent denatures membrane proteins (Bachmann, 1975). The DNA double helix becomes distorted because UV light causes the formation of pyrimidine dimers which then block DNA replication and transcription leading to the bacteria’s death (Guerrero-Beltran and Barbosa-Canovas, 2004; Lado and Yousef, 2002). UV irradiation is a non-thermal process that can reduce the presence of pathogens (Sumner at al., 1996) while not significantly affecting the color or rancidity of fresh poultry (Wallner-Pendleton et al., 1994). Another benefit to the poultry processor is that UV irradiation does not produce chemical or radiation residues on the meat (Anonymous, 1999b). Several studies have shown UV irradiation to be an effective bactericide on meat (Stermer et al., 1987; Kim et al., 2002) and poultry skin (Wallner-Pendleton et al., 1994; Sumner et al., 1996). Wallner-Pendleton et al., (1994)
and Sumner et al., (1996) reported a 61% and 80.5% reduction respectively in *S. typhimurium* on chicken meat with skin. However, Stermer et al. (1987) reported an approximate 2 log reduction in bacteria from fresh beef.

There have not been many studies involving UV irradiation to reduce levels of *L. monocytogenes* on fresh skinless-boneless chicken breasts. A reduction in *L. monocytogenes* on raw chicken entering a poultry further-processing plant could reduce the persistence of the pathogen in the plant environment and reduce the potential contamination of cooked poultry. The objectives of this research were to evaluate the effects of UV irradiation on the recovery of *L. monocytogenes* on fresh boneless-skinless chicken breasts. In addition, the effects of UV irradiation on the color of breast fillets were also determined.

**Materials and Methods**

**LISTERIA MONOCYTOGENES CULTURES & FILLET INOCULATION**

Four strains of *L. monocytogenes*, each with different antibiotic resistance profiles, were used for this experiment. The 4 strains were chosen from 161 isolates that were originally isolated from a poultry further-processing plant and stored in the culture collection of the USDA-Agricultural Research Service-Bacterial Epidemiology and Antimicrobial Resistance Research Unit located in Athens, GA. The 4 strains chosen each had different antibiotic resistance profiles to 4 specific antibiotics (ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline) as determined by the microdilution method and described by Trek Diagnostic Systems, Inc.². Susceptibility

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² Trek Diagnostic Systems, Inc., Cleveland, OH 44131
breakpoints were established by Clinical and Laboratory Standards Institute\(^3\) (CLSI) for Gram-positive organisms. The 4 strains were labeled J, L, N, and X. Strain J was intermediate / resistant to both ceftriaxone and oxacillin with 2% NaCl (MIC 16 / \(\geq\) 32 and \(\geq\) 4, respectively) and sensitive to both ciprofloxacin and tetracycline (MIC \(\leq\) 1 and \(\leq\) 4, respectively). Strain L was intermediate / resistant to both ceftriaxone and tetracycline (MIC 16 / \(\geq\) 32 and 8 / \(\geq\) 16, respectively) and sensitive to both oxacillin with 2% NaCl and ciprofloxacin (MIC \(\leq\) 2 and \(\leq\) 1, respectively). Strain N was intermediate / resistant to 3 antibiotics; ceftriaxone, oxacillin with 2% NaCl, and ciprofloxacin (MIC 16 / \(\geq\) 32, \(\geq\) 4, and 2 / \(\geq\) 4, respectively) and sensitive to tetracycline (MIC \(\leq\) 4). Strain X was intermediate / resistant to all 4 antibiotics: ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline (MIC 16 / \(\geq\) 32, \(\geq\) 4, 2 / \(\geq\) 4, and 8 / \(\geq\) 16, respectively).

Cultures of the 4 strains were maintained on BHI\(^4\) agar slants and stored at 4C for inoculation of the raw, skinless, boneless chicken breasts.

The day prior to inoculation, a BHI slant (stored for maintenance at 4C) containing the desired \(L.\ monocytogenes\) strain (J, N, L, or X) for that week was selected with a sterile needle, placed in a 5 ml BHI broth tube, and incubated at 35C for 24 h. After 24 h incubation, the inoculum contained around 1.0 x 10\(^9\) colony-forming units (CFU) per ml. One serial dilution of the original inoculum was then prepared in a 9 ml PBS dilution blank. Ten \(\mu\)l from the dilution blank was placed on the center of each breast (skin side). Using this concentration, each breast received 10\(^6\) CFU/ml. The dilution blank with the inoculum remained on ice throughout the entire procedure. The inoculum was spread evenly across the surface of each breast with a sterile bioloop.

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\(^3\) Clinical Laboratory Standards Institute, Wayne, PA 18439

\(^4\) Criterion, Hardy Diagnostics., Santa Maria, CA 93455
UV EQUIPMENT

A shortwave UV lamp\(^5\) with 2 bulbs generating 254 nm of wavelength, 115 V, 60 Hz, and 0.68 Amps was used in this experiment. The lamp was fitted with adjustable shelves for different levels of exposure. The entire unit was placed in a biohood with a clear glass sliding door which was UV light impenetrable but which still allowed easy access to the UV unit. UV intensity was measured in microwatts per centimeter squared (\(\mu\)W/cm\(^2\)) using an UV intensity meter\(^6\). The shelves were adjusted so that each sample’s inoculated surface received a UV intensity of 1,000 \(\mu\)W/cm\(^2\). UV intensity (1,000 \(\mu\)W/cm\(^2\)) at the expected location for the breast’s surface was measured before and after each trial for accuracy and repeatability.

UV TREATMENT PROCEDURE

Fresh, raw boneless and skinless chicken breast fillets were purchased from a commercial supermarket. To reduce variation, packages with 3 breast fillets from the same brand and processing plant (USDA plant number), use-by-date, and with similar package weights (mean package weight 630 g) were purchased. The study was replicated for 3 weeks for each strain (12 weeks total).

Twenty breasts were inoculated (10 for UV treatment group and 10 were for the non-UV treatment group) and 1 breast (negative control) was tested for the initial presence of \(L.\) monocytogenes. Five trials were conducted each week. Each trial consisted of 2 UV treated breasts and 2 non-UV treated breasts (5 trials x 2 breasts for UV treatment x 2 breasts for control = 20 breasts).

\(^5\) Ultra-Violet Products, Inc., Model XX-15S, Upland, CA 91786
\(^6\) Ultra-Violet Products, Inc., Model J-225, Blak-Ray\(^\circledR\), Upland, CA 91786
Following inoculation, the fillets were allowed to rest at room temperature (~25°C) for 5 min to allow the inoculum to dry. Two of the 4 breasts were chosen at random and put on the UV tray for 5 min of UV treatment. Breasts in the control group remained at room temperature (~25°C) for the 5 min that the UV treatment was occurring. Following treatment, the fillets were aseptically placed into individual sterile bags and stored for 24 h at 4°C. The 1 uninoculated breast (negative control) for initial *L. monocytogenes* presence was also placed in a sterile bag for 24 h at 4°C.

After 24 h, 50 ml sterile PBS was placed into each bag and vigorously shaken by hand for 60 s. Serial dilutions were made at 10⁻², 10⁻³, and 10⁻⁴ for the controls and the UV treated group had dilutions 10⁻¹, 10⁻², and 10⁻³ while the uninoculated breast rinse (negative control) was diluted to 10⁻¹. From each dilution 0.1 ml was spread plated onto duplicate MOX⁷ agar plates for enumeration. MOX plates were incubated for 24 h at 35°C and typical *L. monocytogenes* colonies were recorded as CFU/breast.

**COLOR EVALUATION**

Thirty post-chill broiler carcasses were obtained from a local poultry processing plant. The right and left breast fillets were removed from each carcass. Half of the breasts (30) were irradiated with UV light at a dose of 1,000 µW/cm² for 5 min. The 30 treated and the 30 control fillets were individually bagged. Breast meat color was determined using a reflectance colorimeter⁸ and expressing color using the CIE LAB system of lightness (L*), redness (a*), and yellowness (b*).

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⁷ Oxford Medium Base plus Modified Antimicrobial Supplement, Remel., Lenexa, KS 66220
⁸ Minolta Chroma Meter, Model CR-300, Minolta Co., Ltd., Ramsey, NJ 07446
The colorimeter was calibrated and the breast fillets read through the bag material at three different locations on the skin side at day 0 and then again at day 7. Between days 0 and 7, breasts were stored in the dark at 4C.

STATISTICAL ANALYSIS

For *L. monocytogenes* enumeration, reported values for each strain of CFU/breast were averages (30 observations per mean) from the 3 independent weeks, converted to logarithmic (base 10) units. Five trials of 4 breasts (2 control and 2 UV-treated) performed during 3 separate weeks for each of the 4 different strains equaled 240 total samples. *L. monocytogenes* numbers recovered were log transformed; means were used in statistical analysis. For the color values, reported values were averages of the 3 readings from each breast taken at day 0 and day 7 (30 observations per mean).

Statistical tests were performed by SAS 9.1 (SAS Institute Inc., 2006). The General Linear Models (GLM) procedure was used to analyze both the *L. monocytogenes* recovery (for each subtype) and the color portion (treatment; UV versus control and storage; 0 versus 7 days) of this study.

**Results and Discussion**

ENUMERATION OF *LISTERIA MONOCYTGENES*

Ultraviolet irradiation at a dose of 1,000 µW/cm² for 5 min was effective in decreasing the population of *L. monocytogenes* on raw boneless skinless chicken breasts (Table 1). All 4 strains subjected to UV irradiation showed a highly significant (*P* < .0001) Log₁₀ CFU/breast reduction compared to the control group.
The most antibiotic resistant strain, X, (intermediate / resistant to all 4 antibiotics) showed a reduction of 2.07 Log\textsubscript{10} CFU/breast (99% kill) after UV irradiation. Strain N (intermediate / resistant to ceftriaxone, oxacillin with 2% NaCl, and ciprofloxacin and sensitive to tetracycline) showed only a 1.61 Log\textsubscript{10} CFU/breast reduction (close to 99% kill) in \textit{L. monocytogenes}. The strain most sensitive to the antibiotics tested, J, (intermediate / resistant to both ceftriaxone and oxacillin with 2% NaCl and sensitive to both ciprofloxacin and tetracycline) showed a 1.88 Log\textsubscript{10} CFU/breast decrease (close to 99% kill) in \textit{L. monocytogenes} post UV irradiation. Strain L (intermediate / resistant to ceftraixone, oxacillin with 2% NaCl, and tetracycline and sensitive to ciprofloxacin) showed a Log\textsubscript{10} CFU/breast reduction of 2.04 (99% kill) in \textit{L. monocytogenes}. No \textit{L. monocytogenes} were recovered from the negative control breasts. Therefore, the use of MOX agar was appropriate because it eliminated the background flora, while allowing the inoculated strain to be recovered. If breasts from the grocery store did yield initial \textit{L. monocytogenes}, then MOX agar would not have been effective at differentiating between the inoculated strain and the initial strain.

The potential use of UV irradiation as a bactericidal food safety process for fresh meats, including poultry, is well documented (Stermer et al., 1987; Wallner-Pendleton et al., 1994; Sumner et al., 1996; Kim et al., 2002). The UV dose (1,000 µW/cm\textsuperscript{2} for 5 min) used in this study was an effective bactericide and is in agreement with data published by Berrang et al. (1995). In that study, the same dosage was used to significantly reduce \textit{S. typhimurium} on hatching eggs after a drop suspension inoculation. The 2 log reduction in CFU from UV irradiation in this study supports the findings of Stermer et al. (1987) who evaluated surface bacteria on fresh beef round streak. The 2 log reduction of \textit{L.}
*L. monocytogenes* on raw skinless chicken breast fillets reported in the current study, however, is much greater than that previously reported for *S. Typhimurium* on poultry (Wallner-Pendleton et al., 1994; Sumner et al., 1996). Wallner-Pendleton et al. (1994) noted only a 61% reduction (0.50 Log_{10}) in *S. Typhimurium* on UV treated chicken halves compared to untreated chicken halves after a very high dose of UV light at 82,560\(\mu\)W/cm\(^2\) for 1 min. Sumner et al. (1996) reported a mean reduction of 80.5% on chicken skin after a UV dose of 2,000 \(\mu\)W \cdot s \cdot cm\(^{-2}\).

The reductions in *L. monocytogenes* for the present study are also much greater than those reported by Kim et al. (2002). The authors of that study showed only 0.48 Log\(_{10}\) CFU/cm\(^2\) reduction on chicken meat with skin and a 0.46 Log\(_{10}\) CFU/cm\(^2\) reduction on chicken meat without skin after a UV dose of 500\(\mu\)W/cm\(^2\) for 3 min. One reason for a lower reduction in the Kim et al. (2002) study could be that the inoculum was able to move beneath the surface of the meat where UV loses its effectiveness as a bactericidal agent. In that study, breasts were autoclaved and then the breasts were submerged in a broth with \(10^{9}\) CFU/ml *L. monocytogenes*. Once submerged in the broth, *L. monocytogenes* could have moved throughout the interior of the muscle and was then shielded from the UV light. UV only has bactericidal effects on the surface. This characteristic of UV light was noted by Stermer et al. (1987). The authors reported that “rough” cut meat submerged in a broth inoculate enabled the pathogen to penetrate into the fibers and become “shielded” from the UV exposure.

Yousef and Marth (1988) reported a decrease of 7 orders of magnitude (>99% kill) of *L. monocytogenes* on tryptose agar. This illustrates the fact that UV light has limited ability to penetrate and is most effective when applied to smooth surfaces.
(Stermer et al., 1987). This scenario was also observed by Kim et al. (2002). Although the authors noted no significant reduction of *L. monocytogenes* on chicken meat, they did note a significant (*P* < 0.05) reduction (4 logs) of *L. monocytogenes* on the smooth surface of stainless steel chips after UV irradiation. A possible explanation for less dramatic reductions of *L. monocytogenes* on chicken breasts in this study than on those for studies involving smooth surfaces is the irregular surface of the meat and protein membranes. Any cutting / pulling during deboning could result in small breaks or tears in the surrounding membrane and uneven rough surfaces of the muscle fibers in these areas. These breaks or tears in the muscle could allow *L. monocytogenes* protection from surface UV exposure.

The 2 log reduction achieved in this study not only was statistically significant but was also biologically significant. The 10^6 CFU/ml inoculation load, used in this study, was more numerous than what would be expected to occur naturally on fresh breasts. Instead, the expected load of *L. monocytogenes* on raw breast fillets would be around 10^1 to 10^2 CFU. Therefore, the 2 Log_{10} CFU/breast reduction by UV irradiation obtained in this study could possibly eliminate all the *L. monocytogenes* cells on the surface effectively stopping raw poultry meat as a vector for the pathogen’s entry into a poultry further-processing plant

L*, a*, and b* COLOR SPACE VALUES

UV irradiation had only minor effects on color (Table 2). For the 0 sampling day there were no significant differences in any of the color values due to the UV treatment. After 7 days of storage at 4C, the UV treated breast fillets were significantly less red (a*
values 0.39 to 0.63, respectively). The UV treated breasts after 7 days of storage exhibited higher yellowness (b*) values compared to the control breasts, 3.91 to 3.28 (P = 0.07). The lightness (L*) values after 7 days of storage were almost identical between the UV treated and control breasts, 56.93 to 56.57, respectively. Overall, the effects of the UV treatment on color was minimal, and the difference in a* values (redness) after 7 days of storage would probably not be noticeable by the poultry further-processor and/or the consumer. These results are in agreement with Wallner-Pendleton et al., (1994) and Stermer et al., (1987) who studied the effects of UV irradiation with chicken meat and beef.

The overall breast lightness values for both the UV treated group and the control group combined significantly (P < 0.05) increased over the 7 day period in dark cold storage compared to day 0. This is in agreement with the findings of Petracci and Fletcher (2002). The yellowness and redness color values did not change significantly (P > 0.05) for both the UV treated and untreated group combined after 7 days in dark cold storage compared to day 0. The redness values decreased and the yellowness values increased on the breast fillets for both treatment groups slightly over the 7 day storage period. These trends in changes of color over time are all in agreement with the results from Petracci and Fletcher (2002).

In summary, a UV dose of 1,000 µW/cm² for 5 min applied to the surfaces of raw boneless skinless chicken breast fillets significantly reduced the recovery of L. monocytogenes. The UV irradiation process did have a small significant effect on redness 7 days after UV exposure. However, this change would probably not be large enough to be noticed visually. Thus, a UV irradiation process could be administered in
the raw poultry processing plant immediately prior to packaging to significantly lower *L. monocytogenes* on the raw breast filets shipped to a poultry-further processing plant. This could decrease the amount of *L. monocytogenes* entering a poultry further-processing plant, and lessen the chances for contamination of a cooked poultry product.
References


Anonymous, 1999b. USDA issues final rule on meat and poultry irradiation.


Table 4.1. Mean and standard error of the mean Log_{10} CFU/breast values of 4 strains of *Listeria monocytogenes* for UV treated and untreated breast fillets

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Control</th>
<th>UV treated</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>6.40 ± .02</td>
<td>4.52 ± .08</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>L</td>
<td>6.35 ± .03</td>
<td>4.31 ± .12</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>N</td>
<td>6.31 ± .02</td>
<td>4.70 ± .10</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>X</td>
<td>6.09 ± .03</td>
<td>4.02 ± .11</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>

n = 30

<sup>1</sup>*L. monocytogenes* strains based on antibiotic resistance profiles:
Table 4.2.  L*, a*, and b* color space values\(^1\) (mean ± std error) of UV treated\(^2\) and untreated raw skinless boneless chicken breast fillets

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>L* (lightness)</th>
<th>a* (redness)</th>
<th>b* (yellowness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>53.32 ± .8</td>
<td>1.50 ± .1</td>
<td>1.13 ± .3</td>
</tr>
<tr>
<td>0</td>
<td>UV</td>
<td>55.31 ± .6</td>
<td>1.40 ± .1</td>
<td>1.73 ± .2</td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>0.07</td>
<td>0.59</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>56.57 ± .5</td>
<td>0.63 ± .1</td>
<td>3.28 ± .2</td>
</tr>
<tr>
<td>7</td>
<td>UV</td>
<td>56.93 ± .3</td>
<td>0.39 ± .1</td>
<td>3.91 ± .2</td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>0.56</td>
<td>0.05</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^1\)Values are means (± std error) with 30 observations per mean.  Color values were noted immediately after UV irradiation (day 0) and 7 days later after being held at 4C.

\(^2\)UV irradiation was administered at a dose of 1,000 \(\mu W/cm^2\) for 5 min.
CHAPTER 5
SUMMARY AND CONCLUSION

The purpose for this study was to test the bactericidal properties of UV light could be applied to raw breast fillets immediately prior to packaging at the slaughter plant as a way to reduce *L. monocytogenes* from entering a poultry further processing plant. The experiments presented were performed to address three objectives: 1) To determine the effect that UV irradiation of raw chicken breast fillets has on the recovery of *L. monocytogenes*, 2) To determine if UV irradiation selected for increased antibiotic resistance among the surviving *L. monocytogenes*, and 3) To determine if any undesirable changes of the meat color occurred from UV irradiation.

For the first objective, 4 different strains of *L. monocytogenes* were used based on differing subtypes and antibiotic resistance profiles. Two populations of breast fillets were compared, a UV treated group (UV dose of 1,000μW/cm² for 5 min) and non UV treated group. Both groups were inoculated with *L. monocytogenes*. After treatment or non treatment, the breasts were stored for 24 h at 4°C to simulate shipment from the slaughter plant to the cook plant. Breast fillets were then rinsed with 50 ml PBS and the rinsate was used to recover *L. monocytogenes* by the spread plate method for enumeration. All 4 strains subjected to UV irradiation showed a highly significant (P < 0.0001) Log₁₀ CFU / breast reduction compared to the non treatment group. A 2 log reduction (~99% kill) in *L. monocytogenes* was observed in the UV treated group compared to the non treated group. In a real world scenario, raw deboned fillets would
have around 10 – 100 cells of *L. monocytogenes* present on their surface prior to packaging at the slaughter plant. Therefore, the 2 log reduction achieved by UV irradiation could possibly eliminate all *L. monocytogenes* cells on the surface of the raw fillets.

For the second objective, the microdilution method was performed to determine the antibiotic resistance profiles of the UV treated and the non treated fillets. The UV treatment, at a dose of 1,000µW/cm² for 5 min had no significant effect (P values > 0.05) on the antibiotic resistance profiles of all 4 *L. monocytogenes* strains tested. Both strains J and N showed no changes in their antibiotic resistance profiles between the UV treated group and the non treated group to ceftriaxone, oxacillin with 2% NaCl, ciprofloxacin, and tetracycline. Strain L for the UV treated group was noted to have small non significant changes in both ceftriaxone and oxacillin with 2% NaCl compared to the non treatment group. UV treated isolates of subtype X had a small non significant change in resistance to oxacillin with 2% NaCl and a 20% decrease (P = 0.07) in resistance to ciprofloxacin compared to the non treated group. This evidence shows that UV irradiation, as an antimicrobial food process, not only kills bacteria but does not select for more drug resistant survivors with *L. monocytogenes*.

For the third objective, lightness (L*), redness (a*), and yellowness (b*) color values were obtained from UV irradiated fillets and non treatment fillets by the CIELAB color space system. UV irradiation at a dose of 1,000µW/cm² for 5 min had only a slight effect on meat color. There were no significant differences (P > 0.05) in lightness and yellowness values at days 0 and 7 between the UV treated fillets and the non treated fillets. The UV treatment did have a significant effect (P < 0.05) on redness values at day
7. The UV treated fillets were lower in redness values than the non treated fillets after 7 days of storage at 4°C, however, at day 0, there was no significant difference (P > 0.05) in redness values between the UV treated and non treated groups. Customers and further processors would not be able to distinguish visibly between which fillets were UV irradiated and which fillets were not treated.

In conclusion, these data suggest that a UV dose of 1,000µW/cm² for 5 min applied to the surfaces of raw boneless skinless chicken breast fillets was effective at reducing the presence of *L. monocytogenes* without selecting for increased antibiotic resistance among the survivors and without negatively affecting the color of the meat. UV irradiation could be applied as an antimicrobial food process immediately prior to packaging at the slaughter plant to eliminate raw poultry as a vector for *L. monocytogenes* entrance into a poultry further processing plant. This, in turn, could possibly reduce persistence of this pathogen in the further processing plant and reduce chances of contaminating a cooked product. Reducing *L. monocytogenes* entry, persistence, and contamination of foods could impact listeriosis outbreaks due to consumption of contaminated poultry products.