

Addressing and anticipating food safety challenges:  
Microbiology and policy frameworks for  
Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella*

by

Daniel Alan Unruh

B.S., Kansas State University, 2012  
B.S., Kansas State University, 2012  
M.S., University of Nebraska-Lincoln, 2014

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## Abstract

Food safety is a public health issue that demands coordinated scientific and policy solutions. Despite advancements in interventions and surveillance, Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. continue to cause outbreaks in a wide variety of food products. In light of these public health urgencies, both microbiological and policy frameworks are needed to address and anticipate future food safety challenges related to these pathogens. Laboratory-based techniques are used to address (1) whether common processing stresses change the susceptibility of STEC and *Salmonella* to food-grade antimicrobials, (2) whether differences in STEC attachment to beef tissue can inform intervention strategies, and (3) the efficiency of a combined sanitizer approach to reduce *Salmonella* on spinach. *Salmonella* Montevideo, Newport, and Typhimurium, and STEC O26, O45, O103, O111, O145, and O157:H7 were subjected to salt, acid, heat, freeze-thaw, alkaline and no (control) stress, and then challenged with the antimicrobials lauric arginate, citric acid plus hydrochloric acid, peroxyacetic acid plus acetic acid and hydrogen peroxide, lactic acid plus citric acid, and lactic acid. Growth/inhibition/no-growth was determined by absorbance values. While differences ( $p \leq 0.05$ ) were observed between some of the stressors and controls, the minimum inhibitory concentrations (MICs) observed for both STEC and *Salmonella* were below maximum concentrations permitted by the United States Department of Agriculture (USDA). STEC serogroups were grown in nutrient-dense or nutrient-limiting media and inoculated onto lean or adipose, pre-rigor (warm) or chilled beef tissue. Loosely and firmly attached cells were plated onto MacConkey agar at several time points. When grown in nutrient-dense media, time  $\times$  sample type (buffer versus homogenized sample) and sample type  $\times$  tissue type (adipose versus lean) were significant ( $p < 0.001$ ). For nutrient-limited cells, tissue type was a significant main

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Approved by:

Co-Major Professor  
Sara E. Gragg

Approved by:

Co-Major Professor  
Justin J. Kastner

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## List of Abbreviations and Symbols

|                 |                                                                   |
|-----------------|-------------------------------------------------------------------|
| 9/11            | September 11, 2001 terrorist attack on the U.S.                   |
| %               | percent                                                           |
| °C              | degrees Celsius                                                   |
| A/E             | attaching and effacing lesion                                     |
| ABR             | antibiotic resistance                                             |
| AMR             | antimicrobial resistance                                          |
| APHIS           | Animal and Plant Health Inspection Service                        |
| ATF             | Bureau of Alcohol, Tobacco, Firearms, and Explosives              |
| ATR             | acid tolerance response                                           |
| $a_w$           | water activity                                                    |
| CAFO            | concentrated animal feeding operation                             |
| CaliciNet       | National Electronic Norovirus Outbreak Network                    |
| CBRNE           | chemical, biological, radiological/nuclear, and explosive hazards |
| CDC             | Centers for Disease Control and Prevention                        |
| CEPH            | Council on Education for Public Health                            |
| CFR             | <i>Code of Federal Regulations</i>                                |
| CFSAN           | FDA Center for Food Safety and Applied Nutrition                  |
| CFU             | colony forming unit                                               |
| CIP             | Center for Infrastructure Protection and Homeland Security        |
| cm <sup>2</sup> | centimeters squared                                               |
| Codex           | Codex Alimentarius Commission                                     |
| CRISPRs         | clustered regularly interspaced short palindromic repeats         |
| CSP             | cold shock protein                                                |
| CSPI            | Center for Science in the Public Interest                         |
| CT-SMAC         | cefixime-tellurite sorbitol MacConkey agar                        |
| CVM             | FDA Center for Veterinary Medicine                                |
| DNA             | deoxyribonucleic acid                                             |
| DNB             | Dey-Engley neutralizing broth                                     |
| DOD             | Department of Defense                                             |
| ECDC            | European Centre for Disease Control and Prevention                |
| ECMWF           | European Centre for Medium-Range Weather Forecasts                |
| EFSA            | European Food Safety Authority                                    |
| EHEC            | Enterohemorrhagic <i>Escherichia coli</i>                         |
| EPA             | Environmental Protection Agency                                   |
| EPC             | extracellular polymeric substances                                |
| EPIA            | <i>Egg Products Inspection Act</i> (1970)                         |
| EPIS            | Epidemic Intelligence Information System                          |
| EU              | European Union                                                    |
| FA              | Food and Agriculture Critical Infrastructure                      |
| FBI             | Federal Bureau of Investigation                                   |

|                   |                                                                   |
|-------------------|-------------------------------------------------------------------|
| FDA               | Food and Drug Administration                                      |
| FDCA              | <i>Federal Food Drug and Cosmetic Act (1938)</i>                  |
| FDOSS             | Foodborne Disease Outbreak Surveillance System                    |
| FEMA              | Federal Emergency Management Agency                               |
| FIFRA             | <i>Federal Insecticide, Fungicide, and Rodenticide Act (1972)</i> |
| FMIA              | <i>Federal Meat Inspection Act (1906)</i>                         |
| FoodCORE          | Foodborne Disease Centers for Outbreak Response Enhancement       |
| FoodNet           | Foodborne Disease Active Surveillance Network                     |
| FOOD Tool         | Foodborne Outbreak Online Database                                |
| FSIS              | Food Safety Inspection Service                                    |
| FSMA              | <i>Food Safety Modernization Act (2011)</i>                       |
| FSVP              | Foreign Supplier Verification Programs                            |
| FWD               | Food- and Waterborne Diseases and Zoonoses Surveillance Network   |
| g                 | gram                                                              |
| GAO               | Government Accountability Office                                  |
| GFN               | Global Foodborne Infections Network                               |
| GFS               | U.S. Global Forecast System                                       |
| GIS               | geographic information systems                                    |
| GMPs              | good manufacturing practices                                      |
| h                 | hour                                                              |
| HACCP             | Hazard Analysis and Critical Control Points                       |
| HARPC             | Hazard Analysis and Risk-Based Preventative Controls              |
| HAV               | hepatitis A virus                                                 |
| HEV               | hepatitis E virus                                                 |
| HHS               | Department of Health and Human Services                           |
| HSARPA            | Homeland Security Advanced Research Projects Agency               |
| HSPs              | heat shock proteins                                               |
| HSPDs             | Homeland Security Presidential Directives                         |
| HUS               | hemolytic uremic syndrome                                         |
| IAFP              | International Association for Food Protection                     |
| IFSAC             | Interagency Food Safety Analytics Collaboration                   |
| IPPC              | International Plant Protection Convention                         |
| ISAC              | Information Sharing and Analysis Center                           |
| IT                | information technology                                            |
| LEE               | locus for enterocyte effacement                                   |
| LHR               | locus of heat resistance                                          |
| log <sub>10</sub> | common logarithm (base 10)                                        |
| LPS               | lipopolysaccharide                                                |
| M9                | M9 minimal salts media                                            |
| MAC               | MacConkey agar                                                    |
| MH                | Mueller-Hinton broth                                              |
| MIC               | minimum inhibitory concentration                                  |
| min               | minute                                                            |

|               |                                                                            |
|---------------|----------------------------------------------------------------------------|
| mL            | milliliter                                                                 |
| MMRW          | <i>Morbidity and Mortality Weekly Report</i>                               |
| mRNA          | messenger RNA                                                              |
| NARMS         | National Antimicrobial Resistance Monitoring System                        |
| NEARS         | National Environmental Assessment Reporting System                         |
| nm            | nanometer                                                                  |
| NNDSS         | National Notifiable Diseases Surveillance Systems                          |
| NOAA          | National Oceanic and Atmospheric Administration                            |
| NORS          | National Outbreak Reporting System                                         |
| NoV           | norovirus                                                                  |
| NRF           | National Response Framework                                                |
| NWP           | numerical weather prediction                                               |
| OHA           | Office of Health Affairs                                                   |
| OHSEC         | Office of Security and Emergency Coordination                              |
| OIE           | International Office of Epizootics                                         |
| OIG           | Office of the Inspector General                                            |
| OMVs          | outer membrane vesicles                                                    |
| PAA           | peroxyacetic acid                                                          |
| PCA           | Peanut Corporation of America                                              |
| PCR           | polymerase chain reaction                                                  |
| PFDA          | 1907 <i>Pure Food and Drug Act</i>                                         |
| PFGE          | pulsed-field gel electrophoresis                                           |
| PPD           | Presidential Policy Directive                                              |
| PPIA          | <i>Poultry Products Inspection Act</i> (1957)                              |
| ppm           | parts per million                                                          |
| PulseNet      | National Molecular Subtyping Network for Foodborne Disease Surveillance    |
| PW            | peptone water                                                              |
| RKI           | Robert Koch Institute                                                      |
| RNA           | ribonucleic acid                                                           |
| rpm           | revolutions per minute                                                     |
| RpoS          | master regulator sigma factor                                              |
| rRNA          | ribosomal RNA                                                              |
| RTE           | ready-to-eat                                                               |
| s             | second                                                                     |
| S&T           | Science and Technology Directorate                                         |
| SAS           | Statistical Analysis Software                                              |
| SBS           | sodium bisulfate                                                           |
| SBS-PAA       | sodium bisulfate - peroxyacetic acid                                       |
| SFI/CSI       | Secure Freight Initiative/Container Security Initiative                    |
| SNA           | social network analysis                                                    |
| SPI           | <i>Salmonella</i> pathogenicity island                                     |
| SPS Agreement | <i>Agreement on the Application of Sanitary and Phytosanitary Measures</i> |
| SSOPs         | sanitation standard operating procedures                                   |

|        |                                                      |
|--------|------------------------------------------------------|
| STEC   | Shiga toxin-producing <i>Escherichia coli</i>        |
| T3SS   | type III secretion system                            |
| TACCP  | Threat Assessment and Critical Control Points        |
| TAL    | thin agar layer                                      |
| tRNA   | transfer RNA                                         |
| TSB    | tryptic soy broth                                    |
| U.S.   | United States of America                             |
| UIN    | Urgent Inquiry Network                               |
| UK     | United Kingdom                                       |
| UN     | United Nations                                       |
| USDA   | United States Department of Agriculture              |
| VACCP  | vulnerability assessment and critical control points |
| WGS    | whole genome sequencing                              |
| WHO    | World Health Organization                            |
| WHO CC | World Health Organization Collaborating Centre       |
| WTO    | World Trade Organization                             |
| XLT-4  | xylose-lysine-tergitol 4 agar                        |

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## **Dedication**

To my wonderful family: Dad, Mom, Jake, Drew, and Jenny.

Thank you for the love, encouragement, support, laughter, and joy.

You are the greatest blessings I have ever received.

*Where it all ends  
I can't fathom my friends  
If I knew I might toss out my anchor...  
So I cruise along  
Always searching for songs  
Not a lawyer, a thief, or a banker.*

Jimmy Buffett  
"Son of a Son of a Sailor"

## Preface

The format of this dissertation is unique. As a means to blend both laboratory- and policy-based research, this dissertation is divided into two parts. Chapters 1, 2, 3, and 4 constitute “Part 1: Microbiology” of the dissertation, which is focused on laboratory-based approaches to anticipate and address future food safety issues. Chapters 5, 6, and 7 constitute the dissertation’s “Part 2: Policy,” which utilizes policy analysis to anticipate and address future food safety issues. Both Part 1 and Part 2 have their own stylistic components, particularly regarding citations. Additionally, both have a corresponding bibliography.

The opportunity to write a dissertation that blends both laboratory- and policy-based concepts is the result of good fortune and open-minded advisors. The author’s inspiration for combining laboratory and policy research may be traced back to his attendance on various *Frontier* field trips during his undergraduate career, which fostered an appreciation for taking multidisciplinary approaches to addressing the food system’s big challenges. Upon the arrival of Dr. Sara Gragg at K-State Olathe in 2013, and at the urging of *Frontier* co-director Dr. Justin Kastner, the unique opportunity was seized to do a lab- and policy-based dissertation. The author is very grateful to Justin and Sara’s willingness to embark on this innovative endeavor.

This dissertation is the final outcome of multiple experiences, projects, learnings, and skills acquired. Since the Fall of 2014, the author has been involved in: (1) coursework, (2) a series of lab research projects, (3) attendance at cutting-edge food safety conferences, including three International Association for Food Protection (IAFP) Annual Meetings, (4) teaching experiences acquired through *Food Law and Regulation* and the United States Department of Agriculture (USDA) Cochran program, (5) food safety and homeland security policy analysis (including a paper presented at George Mason University and published in GMU’s Center for

Infrastructure Protection's invitation-only *CIP Report*, as well as an op-ed for *Food Quality Magazine*), (6) direct supervision and hands-on mentoring of over ten undergraduate student workers, (7) additional mentoring of undergraduate students studying and writing on the history of food safety and public health, and (8) textbook chapters (one food microbiology chapter on hamburger safety for *Food Hygiene and Toxicology in Ready-to-Eat Foods*, and one policy development case study on the *FDA Food Safety Modernization Act* (FSMA) for public health educators affiliated with the Council on Education for Public Health (CEPH)). Finally, (9) the author's extensive involvement in the *Frontier* program, as an undergraduate, "visiting" master's student, and doctoral student, has fostered a deep appreciation and love for multidisciplinary thinking. These nine components of the author's doctoral student experience, coupled with immensely helpful previous work with Shiga toxin-producing *Escherichia coli* (STEC) during his master's, have all directly or indirectly informed this dissertation. At the time of writing, additional publications (*e.g.*, in journals of food microbiology, food safety, et cetera) are anticipated.

# **PART ONE: MICROBIOLOGY**

## **Chapter 1 - Literature Review**

### **1.1. FOODBORNE ILLNESS IN THE UNITED STATES**

#### **1.1.1. Introduction.**

Foodborne illness remains a public health problem in the United States (U.S.). Each year, one-in-six (48 million) Americans contract a foodborne illness (Scharff 2012). It is estimated that each year, 31 known pathogens cause 9.4 million episodes of foodborne illness, resulting in nearly 56,000 hospitalizations and approximately 1,400 deaths (Scallan, Hoekstra et al. 2011). Estimates on the economic burden of foodborne illness place health related costs at \$51-\$78 billion annually, with the most “expensive” foodborne agents being *Salmonella*, *Campylobacter* spp., and norovirus (Scharff 2012). Human disease attributable to food is a global burden causing 582 million cases of illness and 420,000 deaths in 2010 (Kirk, Pires et al. 2015).

#### **1.1.2. Food safety governance in the United States.**

Food safety in the U.S. is governed by 16 federal agencies administering 30 federal laws (United States Government Accountability Office 2017). The principle agencies involved in food safety oversight are the Food and Drug Administration (FDA; a part of the Department of Health and Human Services, HHS) and the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) (Johnson 2014).

Food law and regulation in the United States began in 1906 with the *Pure Food and Drug Act*, passed by Congress to remove dangerous foods and drugs from the marketplace and curtail false labeling and advertising practices (Sanchez 2015). Following a widespread scandal involving sulfanilamide, Congress later passed the *Federal Food Drug and Cosmetic Act*

(*FDCA*; 1938) which is still in effect today (Sanchez 2015). The 2011 *Food Safety Modernization Act* amended the 1938 *FDCA*. It strengthens FDA inspection and compliance authority, provides more scrutiny for imported food safety, gives FDA authority to mandate recalls, enhances partnerships in the U.S. and abroad, and focuses on preventative (instead of reactive) controls (United States Congress 2010, Johnson 2014, Unruh 2014). The *Federal Meat Inspection Act* (FMIA) was passed in 1907 giving regulatory power over meat and poultry slaughter, processing, and inspection to the USDA (Johnson 2014, Sanchez 2015). This, and the *Poultry Products Inspection Act* (PPIA; 21 U.S.C. § 451 *et seq.*) and *Egg Products Inspection Act* (EPIA; 21 U.S.C. 1031 *et seq.*) comprise the USDA “enabling acts” (Sanchez 2015). While the FDA and FSIS are the foremost food safety agencies, several others carry responsibility as well. They are listed in the table below:

**Table 1-1: Food safety agencies and responsibilities**

| <b>Agency</b>                                 | <b>Sub-agency</b>                                       | <b>Responsibility</b>                                                                                                    |
|-----------------------------------------------|---------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| United States<br>Department of<br>Agriculture | Food Safety and Inspection Service                      | Safety of meat, poultry, catfish, and egg products                                                                       |
|                                               | Animal and Plant Health Inspection Service              | Preventing introduction/dissemination of plant pests and livestock diseases                                              |
|                                               | Grain Inspection, Packers and Stockyards Administration | Quality standards and inspection for grain and related products                                                          |
|                                               | Agricultural Marketing Service                          | Quality standards for dairy, fruit, vegetables, and livestock                                                            |
|                                               | Agricultural Research Service                           | Provide scientific research to help ensure that the food supply is safe and secure and regulatory requirements are met   |
|                                               | Economic Research Service                               | Analyses of economic issues affecting food safety                                                                        |
|                                               | National Agricultural Sciences Service                  | Provide statistical data related to food safety                                                                          |
|                                               | National Institute of Food and Agriculture              | Supporting food safety projects in the land-grant university system                                                      |
| Department of Health<br>and Human Services    | Food and Drug Administration                            | Ensuring all domestic foods (except those regulated by FSIS) are safe via CFSAN. Ensures safety of pet food through CVM. |
|                                               | Centers for Disease Control and Prevention              | Preventing the transmission, dissemination, and spread of foodborne illness to protect the public health                 |
| Department of<br>Commerce                     | National Marine Fisheries Service                       | Providing voluntary examinations of seafood for safety and quality                                                       |
| Environmental<br>Protection Agency            |                                                         | Regulating use of certain chemicals and substances, including pesticides                                                 |
| U.S. Department of<br>Transportation          |                                                         | Establishing procedures for safety inspections to help ensure sanitary transport of food                                 |

Adapted from: United States Government Accountability Office (2017)

A more robust discussion about food regulation is found in part two's literature review (chapter 5).

## **1.2. COMMONLY IMPLICATED PATHOGENS**

### **1.2.1. *Escherichia coli*.**

#### **1.2.1.1. Introduction.**

*Escherichia coli* is one of the oldest and most frequently-studied pathogens. The pathogenic forms of *E. coli* became a part of the national vocabulary during the 1993 Jack in the Box outbreak, which served as a watershed moment for food safety in America. Shiga toxin-producing *E. coli* (STEC) have a meandering history from both a scientific and regulatory perspective, which will be told (in an abridged sense) in this review.

In the estimation of foodborne illness by Scallan, Hoekstra et al. (2011), *E. coli* O157:H7 is estimated to cause 63,153 illnesses annually, with a hospitalization rate of 46.2% and a death rate of 5%. Non-O157 STEC cause more illnesses (112,752), but the hospitalization and death rates are lower than O157 (12.8% and 0.3%, respectively). In terms of laboratory-confirmed infections reported to FoodNet, a Centers for Disease Control and Prevention (CDC) epidemiological system, in 2014, there were 445 cases of STEC O157, resulting in 154 hospitalizations and three deaths (Crim, Griffin et al. 2015). For non-O157 STEC, there were 690 cases, 104 hospitalization, and no deaths. The incidence of *E. coli* O157:H7 in 2014 was lower than during 2006-08 while the incidence of non-O157 STEC in 2014 was higher than during 2011-13 (Crim, Griffin et al. 2015).

#### **1.2.1.2. Classification and important characteristics.**

*E. coli* is a Gram-negative, facultative anaerobic rod (Montville and Matthews 2008). Serotyping relies on the O, H, and K antigens. Antigen O is an oligosaccharide linked to lipid A in the lipopolysaccharide components of the cell wall). K is the capsular antigen, and H is the flagellar antigen (Batt 1999, Montville and Matthews 2008).

The categorization of *E. coli* is important for public health officials and microbiologists alike. Montville and Matthews (2008), among others, have categorized *E. coli* into six main categories (Table 1-2).

**Table 1-2: Categories of diarrheagenic *E. coli***

| <b>Category</b>                             | <b>Distinguishing Features</b>                                                                                                                                                                                                                        |
|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enteropathogenic<br><i>E. coli</i> (EPEC)   | Human reservoir; causes severe diarrhea in human; induces lesions for attachment; invade epithelial cells.                                                                                                                                            |
| Enterotoxigenic<br><i>E. coli</i> (ETEC)    | Human reservoir; major cause of infantile diarrhea in developing countries; often implicated in travelers' diarrhea; colonizes the small intestine by fimbrial attachment and produces heat-stable or heat-labile enterotoxins that lead to diarrhea. |
| Enteroinvasive<br><i>E. coli</i> (EIEC)     | Human reservoir; causes nonbloody diarrhea and dysentery; invades and multiplies in intestinal epithelial cells, particularly the colon.                                                                                                              |
| Diffusely Adhering<br><i>E. coli</i> (DAEC) | Causes a mild, nonbloody diarrhea in young children (ages 1-5) but not infants; adherence to cells is random; no toxin production                                                                                                                     |
| Enteroaggregative<br><i>E. coli</i> (EAEC)  | Persistent diarrhea in children and infants; produce a characteristic, aggregative adherence that resembles "stacked bricks" on epithelial cells that is unique compared to other <i>E. coli</i>                                                      |
| Enterohemorrhagic<br><i>E. coli</i> (EHEC)  | Cattle and human reservoirs; produce Shiga-like verotoxins (Stx); causes diarrhea, bloody diarrhea, and sometimes leads to hemolytic uremic syndrome (HUS), a potentially fatal kidney disease                                                        |

Adapted from: Montville and Matthews (2008)

As a point of clarification, the term "Shiga toxin-producing *E. coli*" refers to *E. coli* that have genes encoding one or more Shiga toxins, and is synonymous with verotoxigenic, verotoxin-producing, or verocytotoxigenic *E. coli* (VTEC). EHEC are a subset of STEC that are pathogenic to humans, carrying the *eae* gene (discussed in 2.1.3) and often resulting in hemorrhagic colitis (Tarr, Gordon et al. 2005). A special note must be made regarding *E. coli* O104:H4. This pathogen, which caused an outbreak of foodborne illness in Germany in 2011,

displayed both enterohemorrhagic and enteroaggregative qualities (Muniesa, Hammerl et al. 2012). The outbreak, implicating contaminated raw fenugreek sprouts, was severe, infecting 4000 persons, causing 900 cases of hemolytic uremic syndrome, and killing 54 (Karch, Denamur et al. 2012, Castro, Carvalho et al. 2017).

### 1.2.1.3. Virulence and pathogenicity.

Members of the *Enterobacteriaceae* family, including *Escherichia* and *Salmonella*, share several common virulence factors, as shown in Table 1-3 (Murray, Rosenthal et al. 2013).

**Table 1-3: Virulence factors associated with *Enterobacteriaceae***

| <b>Virulence Factor</b>         | <b>Activity</b>                                                                                                                                                                          |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Endotoxin                       | A part of the Gram-negative cell wall; activates immune response upon cell lysis.                                                                                                        |
| Capsule                         | Hydrophilic system of antigens that repel the hydrophobic phagocyte cell surface and interfere with binding of antibodies to the bacteria.                                               |
| Antigenic phase variation       | Genetic control of the O, K, and H antigens results in expression or non-expression of the antigen to evade antibody detection.                                                          |
| Type III secretion system       | Effector system for delivering virulence factors into eukaryotic cells; a “molecular syringe” of approximately 20 proteins that assist in virulence factor transference.                 |
| Sequestration of growth factors | When <i>in vivo</i> , cells require iron as a growth factor; since iron is bound by heme proteins or iron-chelating proteins, the bacteria are able to use siderophores to chelate iron. |
| Resistance to serum killing     | Capsule prevents antibodies from binding to the cell, permitting the cells to survive in the bloodstream instead of being rapidly cleared.                                               |
| Antimicrobial resistance        | Imprudent antibiotic use leads to resistance factors encoded and transferred among bacteria.                                                                                             |

Adapted from: Murray, Rosenthal et al. (2013)

STEC possess a pathogenicity island known as the *locus of enterocyte effacement* (LEE island), which encodes genes responsible for attaching and effacing (A/E) (Gyles 2007, Franzin and Sircili 2015). Among LEE's genetic makeup are 41 genes that encode for the type III secretion system; *eae*, the gene encoding for the attachment protein intimin; *tir*, the gene encoding the intimin receptor which is translocated into the host cell; and other proteins important to the type III secretion system, including *espA*, *espB*, *espC*, and *espD* (Frankel, Phillips et al. 1998, Castillo, Eguiarte et al. 2005). Attaching and effacing is the primary step in STEC colonization of epithelial cells in the intestine (Frankel, Phillips et al. 1998). A final, important pathogenicity factor is the plasmid *pO157* that encodes for enterohemolysin (*Ehx*), which causes destruction of red blood cells through cell cytoplasm insertion and assists in *E. coli* multiplication by releasing iron from the red blood cells (Mainil and Daube 2005, Castro, Carvalho et al. 2017). EHEC also produces a non-Stx cytolethal distending toxin (Karch, Tarr et al. 2005).

The A/E process begins when *E. coli* contacts epithelial cells, often after ingestion of contaminated food. Proteins expressed by the LEE island assemble the type III secretion system, including the outer membrane protein intimin. The secretion system injects Tir (encoded by *tir* gene) into the host cell, which translocates underneath the cellular membrane, breaking down the actin cytoskeleton of the microvillus. Tir interacts with intimin to adhere, accumulate actin, and utilize cytoskeletal proteins to form a pedestal (Frankel, Phillips et al. 1998, Montville and Matthews 2008). The now-formed A/E lesion has thus structurally changed the epithelial cell and prompted adherence, creating the tense, unyielding pedestal (Gyles 2007). Now, STEC can begin excreting Shiga toxins into epithelial tissue and the bloodstream.

The presence of Shiga toxins in EHEC is a result of a prophage that transported the gene from *Shigella dysenteriae* into the *E. coli* chromosome by transduction (Mizutani, Nakazono et al. 1999). The extracellular cytotoxin produced by *S. dysenteriae* is known as Stx, or Shiga toxin (Tarr, Gordon et al. 2005). Shiga toxins 1 and 2 (Stx1 and Stx2, respectively) are the toxins produced by *E. coli* (Tarr, Gordon et al. 2005). The Stx1 molecule is highly conserved and nearly identical to the Stx of *Shigella* (Takao, Tanabe et al. 1988). Stx2 is 58% similar to Stx1 at the amino acid level and 56% identical at the nucleotide level. Allelic variants include Stx1a, Stx1c, Stx1d, Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g; these variants may have different pathogenic potentials (Tarr, Gordon et al. 2005, Castro, Carvalho et al. 2017). Stx1 and Stx2e are encoded by genes in the chromosome, while the remaining Stx2 variants have been bacteriophage that have integrated into the chromosome (Montville and Matthews 2008).

Shiga toxin is an A<sub>1</sub>B<sub>5</sub> toxin: the B subunit binds to a glycolipid on eukaryotic cell surface while the A subunit inhibits protein synthesis by cleaving the *N*-glycoside bond in rRNA, disrupting the activity of tRNA and stopping peptide chain elongation (Endo, Tsurugi et al. 1988, Tarr, Gordon et al. 2005, Montville and Matthews 2008, Castro, Carvalho et al. 2017). Stxs damage vascular endothelial cells, disrupting homeostasis and likely leading to bloody diarrhea (Montville and Matthews 2008). Analysis of STEC infection victims' tissues have shown that Stx2 has more toxicity to microvascular endothelial cells, and the presence of Stx2 alone or Stx2 and Stx1 (as opposed to Stx1 alone) causes a more serious illness (Louise and Obrig 1995, Ray and Liu 2001, Montville and Matthews 2008).

STEC infections often cause a mild gastroenteritis that resolves. In some cases, the disease can proceed to a hemorrhagic colitis presented by pain and bloody diarrhea. Typically, incubation time is three to four days. Bloody diarrhea will present two days thereafter, with a

resolution of symptoms within four to ten days thereafter without treatment (Murray, Rosenthal et al. 2013). Disease can be caused by as few as ten cells (Moore 2004).

STEC infections have the potential to progress to hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (Boyce, Swerdlow et al. 1995, Gyles 2007, Castro, Carvalho et al. 2017). Often, the evolution from a STEC infection to HUS commonly occurs in the young, elderly, and immunocompromised (Montville and Matthews 2008). The connection between STEC infection and HUS was established when scientists identified a toxin lethal to African green monkey kidney cells in the stools of children with HUS (Karmali, Petric et al. 1983). Shortly thereafter, persons who developed bloody diarrhea after consuming undercooked hamburger had *E. coli* O157:H7 isolated from their stools (Riley, Remis et al. 1983). Thrombotic thrombocytopenic purpura is symptomatically similar to HUS except that it causes more pronounced neurological indications: seizures, strokes, and nervous system deterioration (Boyce, Swerdlow et al. 1995). These severe outcomes of STEC infection may lead to long-term sequelae, including significant kidney damage (Griffin and Tauxe 1991). Approximately half of persons developing HUS will require dialysis and 15% will have chronic kidney failure. Recurrence of kidney problems is not unusual (Siegler, Griffin et al. 1993). Other sequelae includes diabetes, neurological disorders, hypertension, urinary abnormalities, bile stones, and colon stenosis (Karch, Tarr et al. 2005, Castro, Carvalho et al. 2017).

The *E. coli* O104:H4 strain implicated in the 2011 German outbreak was found to have both enteroaggregative characteristics (carried on the pAA plasmid) and the genetic code for Shiga toxin production (carried on a lamboid prophage) (Muniesa, Hammerl et al. 2012). The bacterium also displayed antibiotic resistance, producing an extended-spectrum  $\beta$ -lactamase (Muniesa, Hammerl et al. 2012). The pronounced virulence of *E. coli* O104 is likely explained

by “the specific combination of enhanced adhesion, survival fitness, Stx2 production, and antibiotic resistance of the strain”, achieved through a genome with high plasticity [(Karch, Denamur et al. 2012), p. 843]. It was a “virulence blend” and “the ease with which this combination of two mobile DNA elements can be achieved in *E. coli* suggests that this will not be the last surprise” [(Muniesa, Hammerl et al. 2012), p. 4070].

#### **1.2.1.4. Reservoirs and implicated food products.**

*E. coli*, and of particular concern, STEC, colonize bovine (and other ruminant) gastrointestinal tracts (Wells, Shipman et al. 1991, Gyles 2007). The connection to beef emanated from *E. coli* O157:H7 isolations from dairy cattle implicated in an unpasteurized milk outbreak (Martin, Shipman et al. 1986, Borczyk, Karmali et al. 1987). Over 470 STEC serotypes have been isolated from humans, with traceback analysis linking them to a cattle source; more than 430 isolates have been obtained from cattle sources directly (Beutin, Geier et al. 1993, Beutin, Krause et al. 2004, Gyles 2007). Of the STEC outbreaks occurring between 1998-2008, 55% of *E. coli* O157:H7 and 50% of non-O157 outbreaks have implicated beef (Painter, Hoekstra et al. 2013).

Using multi-year outbreak surveillance data, an interagency collaboration among CDC, FDA, and USDA developed source attribution estimates for 2013 using data from outbreaks occurring 1998-2013 and categorizing by 17 food groups. In the analysis, *E. coli* O157:H7 illnesses were most often linked to vegetable row crops and beef (80% from these categories) (Interagency Food Safety Analytics Collaboration 2017). No illnesses were attributed to eggs, pork, grains, or other commodities. Genetic elements similar to those in the German O104:H4 outbreak have been isolated from a slaughterhouse near the epicenter of the outbreak, leading epidemiologist to surmise that a bovine reservoir may exist (Böhnlein, Kabisch et al. 2016). In

addition to produce, *E. coli* O104:H4 has demonstrated adaption to and survival in meat products, including fermented sausages (Böhnlein, Kabisch et al. 2016).

#### **1.2.1.5. Outbreaks.**

Major STEC outbreaks have made an impact on regulatory and public health actions; for a robust discussion on this reality, see part 2 of the dissertation (Chapter 5; Table 5-1), where recent outbreaks are catalogued.

### **1.2.2. *Salmonella* spp.**

#### **1.2.2.1. Introduction.**

Nontyphoidal *Salmonella enterica* causes 11% of foodborne illnesses in the United States, second only to norovirus. *Salmonella* leads foodborne illness agents in hospitalizations and deaths (35% and 28% of all foodborne illness cases, respectively) (Scallan, Hoekstra et al. 2011). According to a *Morbidity and Mortality Weekly Report* (MMRW) report, FoodNet incidences for various foodborne pathogens in 2014 identified 7,452 cases of *Salmonella*, 2,141 hospitalizations, and 30 deaths. The figures for *Salmonella* Typhmimurium were significantly lower than 2006-08 while *Salmonella* Javiana were higher. Compared to 2011-13, *Salmonella* Infantis cases were more numerous in 2014. Overall, incidence of salmonellosis was unchanged (Crim, Griffin et al. 2015). For each case of *Salmonella* that is culture-confirmed, an estimated 38.6 cases go unreported (Voetsch, Van Gilder et al. 2004). An estimated 93.8 million cases of nontyphoidal *Salmonella* occur each year, with 155,000 succumbing to the illness (Majowicz, Musto et al. 2010). Meanwhile, Kirk, Pires et al. (2015) estimate that, globally, 153 million cases of salmonellosis occur annually, accounting for 57,000 deaths.

### 1.2.2.2. Classification and important characteristics.

*Salmonella* strain identification and nomenclature has a long and confusing history (Ryan, Dwyer et al. 2017). *Salmonella* spp. cause three diseases: enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by nontyphoid microorganisms (D'Aoust, Maurer et al. 2008). The enteric diseases are comprised of typhoid and paratyphoid fever. Typhoid fever is a systemic bacterial disease characterized by “insidious onset of fever, severe headache, malaise” and often diarrhea or constipation [(Anonymous 1995), p. 502]. Paratyphoid fever is similar to typhoid fever but milder and with a lower case fatality rate (Anonymous 1995). The causative agents are *Salmonella* Typhi and *Salmonella* Paratyphi (Anonymous 1995). The mode of transmission for both is food and water contaminated with the feces of patients and carriers (Anonymous 1995). Asymptomatic, chronic colonization has also been noted in the medical literature (Murray, Rosenthal et al. 2013). The beguiling story of Typhoid Mary is one of history’s most vivid examples of the impact of typhoid (Marineli, Tsoucalas et al. 2013). Typhoid fever has largely been controlled in modern societies through the advent of common sanitation measures, and *Salmonella* Typhi and *Salmonella* Paratyphi are strict human pathogens (no food reservoirs) (Murray, Rosenthal et al. 2013). Thus, the uncomplicated enterocolitis and systemic infections by nontyphoid *Salmonella* are more relevant to the modern food microbiologist.

The uncomplicated enterocolitis (*i.e.*, gastrointestinal disease) caused by nontyphoidal *Salmonella* is characterized by disease that manifests 8-72 hours after ingestion and is comprised of self-limiting, non-bloody diarrhea with abdominal pain, both of which can last up to five days before resolving without medical intervention (D'Aoust, Maurer et al. 2008). The third category of disease caused by *Salmonella* are a set of systemic infections from nontyphoid strains that

cause chronic conditions such as aseptic reactive arthritis, Reiter's syndrome, and ankylosing spondylitis (D'Aoust, Maurer et al. 2008).

Until 1966, *Salmonella*'s naming scheme was fluid and unregulated. Usually, naming was based on the disease caused, the animal the *Salmonella* strain had been isolated from, or the geographic location of isolation (Ryan, Dwyer et al. 2017). In 1966, some suggested that a three-species naming system be used: *Salmonella enteritidis*, *Salmonella typhosa*, and *Salmonella choleraesuis* (Ewing 1972). New DNA-DNA hybridization techniques in the 1970s led researchers to propose one species (*Salmonella choleraesuis*) and seven subspecies (Crosa, Brenner et al. 1973). However, the use of "choleraesuis" created problems (it denoted both a species and a serovar), leading to the suggestion that *Salmonella enterica* be used as the species type designation in 1986 (Ryan, Dwyer et al. 2017). This was generally accepted, but debate still ensued over using seven subspecies (some of which were further split into sub-subspecies) and the fear that *Salmonella* Typhi may be overlooked if called by its long name *Salmonella enterica* subsp. *enterica* serovar Typhi. More requests, changes, and updates occurred in the late 1980s and into the 1990s. Finally, in 2002, the Judicial Commission of the International Committee for Systematics of Prokaryotes, in Judicial Opinion 80, declared that the *Salmonella* genus is comprised of two species: *Salmonella enterica* and *Samonella bongori* (Tindall, Grimont et al. 2005, Ryan, Dwyer et al. 2017). *Salmonella enterica* is comprised of six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. Serotyping can also occur by somatic O antigens, flagellar H antigens, and Vi capsular antigens (Ryan, Dwyer et al. 2017).\*

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\* It should be noted that, within *Salmonella*, "serovar" can refer to both the antigenic formula and the assigning of formal names to *S. enterica* subsp. *enterica* (again, chosen based upon "diseases associated with infection, the geographic area of their isolation, or typical habitats" for subspecies I). In publication, the full *Salmonella enterica* is used, then the subspecies name (*Salmonella enterica* subsp. *enterica*), then the serovar (ser.) and the name in nonitalicized Roman letters, such as *Salmonella enterica* subsp. *enterica* serovar Typhi. Then, the name can be condensed in further reference, such as *Salmonella* Typhi or *S. Typhi*. If full antigenic definition has occurred, that can be used as well. For the other five *Salmonella enterica* subspecies, serovars are identified by the subspecies

Nevertheless, confusion remains. *Salmonella enterica* subsp. Typhi causes the dramatic, life-threatening typhoid fever; whereas, *Salmonella enterica* subsp. Typhimurium causes a gastrointestinal disease.

### **1.2.2.3. Virulence and pathogenicity.**

The aforementioned *Enterobacteriaceae* virulence factors (Table 1-3) should be referenced for *Salmonella*. The infectious dose of *Salmonella* may be as low as 30 cells; however, larger titers ( $10^6$ - $10^8$  cells) more commonly result in symptomatic disease (D'Aoust 1994, Murray, Rosenthal et al. 2013). Upon ingestion, *Salmonella* must evade nonspecific host defenses, such as lactoperoxidase in saliva, stomach acidity, and mucoid secretions in intestinal cells (D'Aoust, Maurer et al. 2008). In the small intestine, *Salmonella* attach to mucosa and invade into the M (microfold) cells in Peyer patches or invade enterocytes (Murray, Rosenthal et al. 2013). The membrane ruffling and resultant cell apoptosis of epithelial and phagocytic cells elicits the diarrheal response (D'Aoust, Maurer et al. 2008).

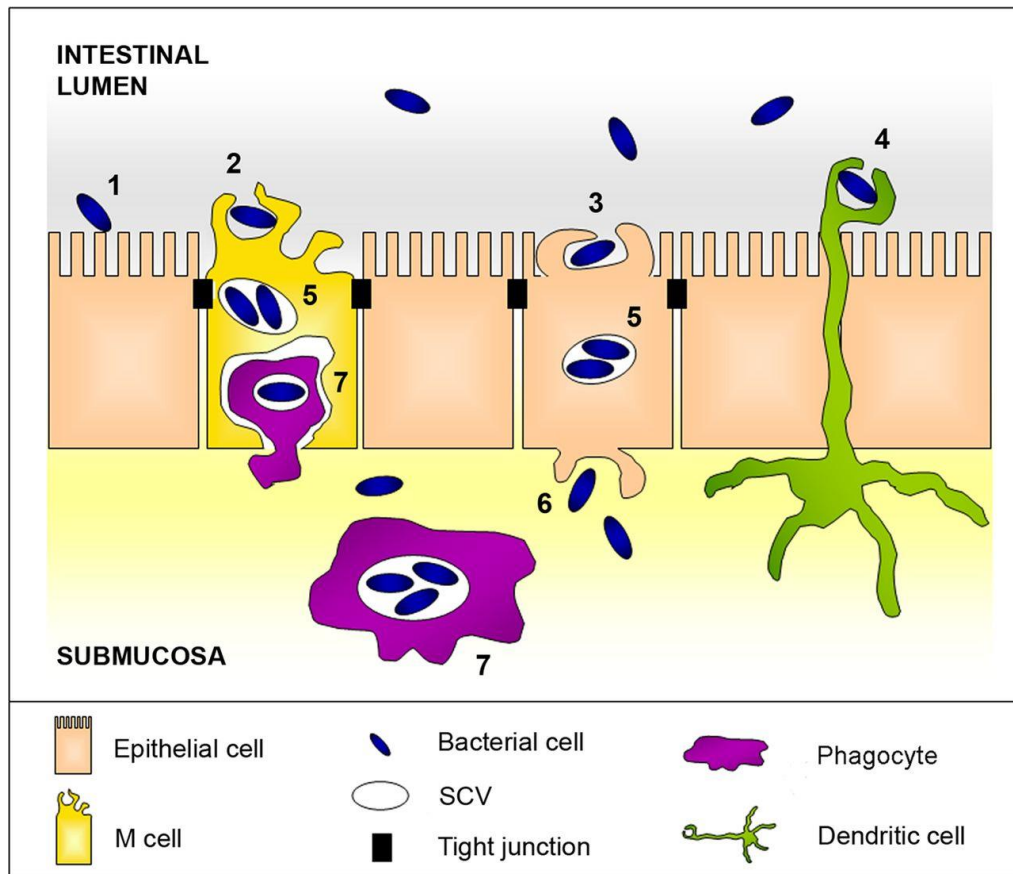
*Salmonella* does not replicate in the host cytoplasm: replication occurs in endocytotic vacuoles which move from the apical to the basal pole of the host cell, where the pathogen is released. During this migration, *Salmonella* must evade host defenses, including phagocytosis and antimicrobial peptides in the cytoplasm. Avoiding phagocytosis may involve mechanical escaping, inhibition of the acidification of the phagosome, and/or prevention of phagosome-lysosome function (D'Aoust, Maurer et al. 2008). *Salmonella* may be released into the blood or lymphatic system, as well (Murray, Rosenthal et al. 2013).

The process of attachment, engulfment, and replication is encoded by gene islands located on the bacterial chromosome: pathogenicity islands I, II, III, IV, and V (*Salmonella*

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name in Roman letters and the antigenic formula: O (somatic) antigens: Vi (when present): H (flagellar) antigens (phase 1): H antigens (phase 2, if present). An example would be *Salmonella* subsp. II 58:l,z<sub>13</sub>,z<sub>28</sub>,z<sub>6</sub>.

pathogenicity islands, or SPIs) (Fàbrega and Vila 2013). Adhesins, which assist in attachment to epithelial cells, are located on SPI 3 and 4. The processes of invasion is reliant upon interplay of invasion proteins (known as salmonella-secreted invasion proteins, or Ssps) that are inserted into the cell by a type III secretion system, both of which are encoded on SPI 1 and 5 (Fàbrega and Vila 2013, Murray, Rosenthal et al. 2013). A second type III secretion system injects proteins that assist in bacterial evasion of host cell responses, and the secretion system and protein genes are located on SPI 2 and 3 (Fàbrega and Vila 2013, Murray, Rosenthal et al. 2013). A virulence plasmid (pSLT) also plays a role, encoding effector proteins that assist in virulence, system spread, macrophage lysing, and iron retrieval (D'Aoust, Maurer et al. 2008, Fàbrega and Vila 2013). Figure 1.1 gives a visual overview of *Salmonella* pathogenesis.



Used with permission from Fàbrega and Vila (2013) and Sansonetti (2002)

**Figure 1.1: Pathogenesis model of *Salmonella enterica*.**

**1:** *Salmonella* cells attach to the intestinal epithelium by means of adhesins, such as those encoded within SPI-3 and SPI-4. **2 and 3:** Invasion of bacteria follows, and engulfment is mediated by virulence factors encoded within SPI-1 and SPI-5. **4:** Alternatively, bacterial cells can also be directly taken up by dendritic cells from the submucosa. **5:** Once inside the cytoplasm, *Salmonella* is localized within the *Salmonella*-containing vacuole (SCV), where it replicates. Factors encoded within SPI-2 and the pSLT (virulence) plasmid are essential for survival. **6:** The SCVs transcytose to the basolateral membrane and release the internal cells to the submucosa. **7:** Bacteria are internalized within phagocytes and located again within an SCV, where SPI-3, in addition to SPI-2 and the pSLT plasmid, play an important role. Lastly, these infected phagocytes can disseminate through the lymph and the bloodstream. [direct figure legend from Fàbrega and Vila (2013)].

#### 1.2.2.4. Reservoirs and implicated food products.

The most common food exposures in North America for *Salmonella* are eggs, poultry, pork, vegetables, and beef (23.3%, 22.6%, 15.5%, 10.1%, and 8.8%, respectively) (Hoffmann, Devleesschauwer et al. 2017). In another study, the Interagency Food Safety Analytics Collaboration (2017) attributed *Salmonella* illnesses to multiple food categories, with 75% of

illnesses attributed to seeded vegetables, eggs, chicken, other produce, pork, beef, and fruits (Interagency Food Safety Analytics Collaboration 2017). In a survey of several nations, including European Union nations, the United States, and Japan, eggs and broiler chickens were the principal food sources, with some attribution to produce as well (Pires, Vieira et al. 2014).

#### **1.2.2.5. Outbreaks.**

Major *Salmonella* outbreaks (like major STEC outbreaks) have prompted the adoption of new laws and regulations; this is explored further in part 2 of the dissertation (Chapter 5; Table 5-2), where recent *Salmonella* outbreaks are highlighted.

### **1.2.3. Other foodborne pathogens of concern.**

#### **1.2.3.1. Viral foodborne pathogens.**

A wide array of viruses can be transmitted through food, representing a diversity of virus families and associated disease (*e.g.*, several enterotropic viruses, including human rotavirus and parvovirus; several neurotopic viruses, including poliovirus, nipah virus, and tick-borne encephalitis virus; pneumotropic viruses, including the human coronaviruses SARS and MERS virus, as well as avian influenza virus; and the headline-grabbing, highly virulent Ebola virus) (Bosch, Pintó et al. 2016). However, the most-commonly implicated and reported foodborne viral diseases are the noroviruses (NoV), hepatitis A virus (HAV), and the emerging threat, hepatitis E virus (HEV) (Bosch, Pintó et al. 2016). The majority of foodborne viruses are transmitted via the fecal-oral route: NoV is easily spread by high titers excreted by hosts prior to symptom presentation, while HAV (of which infection results in lifelong immunity) causes severe infections in humans that travel to or consume food originating from developing regions where the disease is endemic (Bosch, Pintó et al. 2016). A wide variety of foods have been implicated in viral outbreaks, including deli meats, bakery products, ice cubes, berries, and

shellfish (Koopmans and Duizer 2004). Because viruses cannot replicate in food, the amount of contamination cannot increase during normal food handling, packaging, or other processing events; however, viruses are more resistant to the interventions used to control bacteria (Koopmans and Duizer 2004). Thus, proper hygiene and the use of drinking-quality water is the best way to control viral foodborne illness spread (Koopmans and Duizer 2004). Researching NoV has been difficult due to the inability of *in vitro* production of viruses in laboratory settings. Recently, however, scientists have successfully cultivated human NoV in the lab, which may provide new insights that lead to effective control and treatment of norovirus in the future (Ettayebi, Crawford et al. 2016).

#### **1.2.3.2. Bacterial foodborne pathogens.**

A majority of *L. monocytogenes* illnesses are linked to fruits and dairy products; however, estimating the number of illnesses attributed to *L. monocytogenes* is difficult due to the rarity of outbreaks (Interagency Food Safety Analytics Collaboration 2017). *Campylobacter* is frequently implicated in chicken, seafood, and row crop outbreaks. An increase in dairy-based *Campylobacter* outbreaks is attributed to unpasteurized milk, which, although not widely consumed, causes many *Campylobacter* outbreaks (Interagency Food Safety Analytics Collaboration 2017). *Staphylococcus* spp. and *Clostridium* spp. are also foodborne pathogens of concern.

The most recent estimate of foodborne illness attribution and burden was performed by Scallan, Hoekstra et al. (2011). Of the nearly 48 million foodborne illnesses that occur each year, only 20% (9.4 million) are attributable to 31 known pathogens. The remaining 80% are likely emanate from unculturable, unidentifiable, and/or unknown pathogens. Regarding number of illnesses, norovirus leads the rankings with an estimated 5.5 million cases annually (Table 1-4).

Nontyphoidal *Salmonella* causes the most hospitalizations and deaths among the known pathogens (Table 1-4). Unspecified agents include bacteria and viruses that have not been directly linked to food; bacteria, viruses, other microorganisms, or chemicals whose ability to cause illness has not been demonstrated; and agents that simply cannot be identified using current scientific techniques (Scallan, Hoekstra et al. 2011, Centers for Disease Control and Prevention 2016).

**Table 1-4: Estimated contributions of various pathogens to illnesses, hospitalizations, and deaths related to foodborne illness in the United States**

| Rank | Illnesses                        |           | Hospitalizations                 |        | Deaths                           |        |
|------|----------------------------------|-----------|----------------------------------|--------|----------------------------------|--------|
|      | Pathogen                         | Number    | Pathogen                         | Number | Pathogen                         | Number |
| 1    | Norovirus                        | 5,461,731 | <i>Salmonella</i> , nontyphoidal | 19,336 | <i>Salmonella</i> , nontyphoidal | 378    |
| 2    | <i>Salmonella</i> , nontyphoidal | 1,027,561 | Norovirus                        | 14,633 | <i>Toxoplasma gondii</i>         | 327    |
| 3    | <i>Clostridium perfringens</i>   | 965,958   | <i>Campylobacter</i> spp.        | 8,463  | <i>Listeria monocytogenes</i>    | 255    |
| 4    | <i>Campylobacter</i> spp.        | 845,024   | <i>Toxoplasma gondii</i>         | 4,428  | Norovirus                        | 149    |
| 5    | <i>Staphylococcus aureus</i>     | 241,148   | <i>E. coli</i> (STEC) O157       | 2,138  | <i>Campylobacter</i> spp.        | 76     |

Adapted from: Scallan, Hoekstra et al. (2011) and Centers for Disease Control and Prevention (2016)

## 1.3. BACTERIAL STRESS AND INJURY

### 1.3.1. Introduction.

Antimicrobial resistance is considered by many to be a public health crisis. Scientists surmise that “resistance genes circulate between animals, people, and the environment” (Laxminarayan, Duse et al. 2013). The question of whether bacteria can become resistant to antimicrobials often involves a discussion of stress and injury, particularly how stress and sublethal injury may result in resistant microorganisms.

### **1.3.2. Control of pathogens.**

#### **1.3.2.1. Stress and injury.**

In a seminal review on bacterial stress and injury, Hurst (1977) states that the physical and chemical treatments used in food processing are designed to destroy microorganisms; if the treatment, however, does not reach maximum severity, the surviving microbes are *injured*. The physical treatments include heat, cold, drying, freeze-drying, freezing, cold shock, osmotic activity, and irradiation (Hurst 1977). Chemical interventions include starvation, pH manipulation, preservatives, and disinfectants (Hurst 1977).

In another formative text on bacterial stress and injury, Hurst (1984) states that stress and sublethal injury have been used interchangeably to describe the same phenomenon, but argues that “the term injury is preferred because, by analogy with other higher organisms, it evokes an image of temporary and repairable damage”; whereas, stress “has a more subtle meaning and may not be manifested by physical damage, although it can alter the behavior of an individual” [(Hurst 1984), p. 78].

In an excellent review on stress and sublethal injury, Wesche, Gurtler et al. (2009), in reference with the work of Hurst (1977), (Hurst 1984), Gilbert (1984), Murano and Pierson (1993), and Storz and Hengge-Aronis (2000), create a series of definitions that will be used in this Literature Review and are given in Figure 1.2.

**Bacterial injury:** the effect of one or more sublethal treatment on a microorganism.

**Sublethal injury:** exposure to a chemical or physical process that damages but does not kill a microorganism; damage to structures within the cells leading to loss of cell function in a transient or permanent manner.

**Stress:** any departure from optimal conditions with the potential to decrease bacterial growth.

**Bacterial stress:** a physical, chemical, or nutritional condition insufficiently severe to kill, resulting in sublethally injured microbes.

Adapted from Hurst (1977), Hurst (1984), Gilbert (1984), Murano and Pierson (1993) and Storz and Hengge-Aronis (2000).

**Figure 1.2: Definitions for injury and stress related to microorganisms**

Stress is categorized as mild, moderate, or extreme. Mild stress is sublethal: the growth rate stops or is hampered, but viability remains. Moderate stress stops growth and some cell viability is lost. Extreme (severe) stress often results in lethality and only a minor population survives, if there are any survivors at all (Yousef and Courtney 2003).

Stress is a common event for foodborne pathogens. As Lou and Yousef (1996) state:

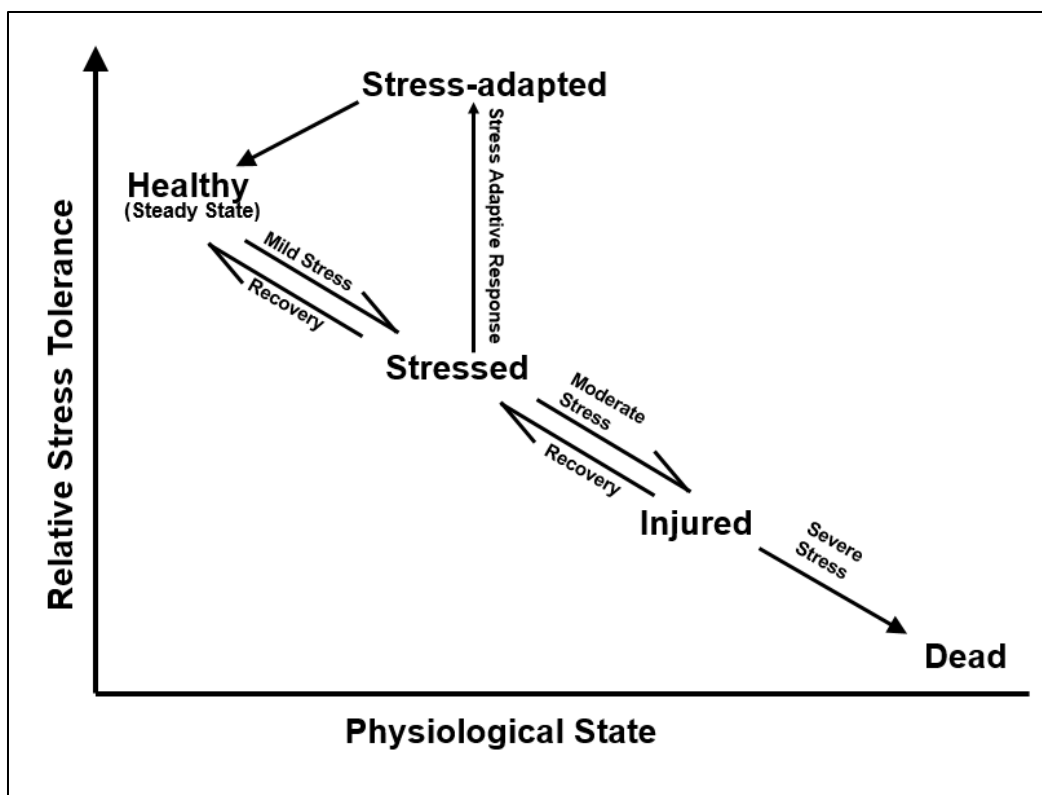
*During processing, microorganisms in the food undergo various kinds of stresses, such as acid stress in cheese, sausage, pickle, yogurt, and sour cream, starvation stress on equipment surfaces and in water, ethanol stress in sufu (mold-fermented soybean curd produced in some Asian countries), osmotic stress in cheese, pickle, and sausage, and oxidation stress in milk when H<sub>2</sub>O<sub>2</sub> is added or when the lactoperoxidase system is activated.* [p.468-469]

It is common to think that food is a luxurious environment for microbial growth. In actuality, it is a milieu “containing complex substrates for growth, competition in the form of other bacteria, a lack of moisture perhaps, and acids and other by-products that may have been produced by competitors” [(Archer 1996), p. 92]. Indeed, foodborne bacteria encounter intrinsic

(*i.e.*, the food matrix itself) and extrinsic (*e.g.*, water activity, pH, temperature) stressors (Buchanan and Doyle 1997, Chung, Bang et al. 2006). Some surmise that adaptation to sublethal stress results in protection against the same stress later, even if it is more severely applied (Lou and Yousef 1996, Yousef and Courtney 2003). Others have shown that a certain stress may confer cross protection against other stresses (Leyer and Johnson 1993, Lou and Yousef 1996). Injured cells may not be detected and remain in the food product and make people sick at a later time (Montville and Matthews 2008).

For the purpose of this literature review, both *stress* and *injury* are used to define a physical, chemical, or microbiological challenge toward bacteria. It is often stress that leads to injury, and not vice versa (see Figure 1.3). While the terms are not explicitly used interchangeably, both may result in the physiological changes that lead to adaptation and/or resistance, and thus, both terms are found in this Literature Review. However, the term *stress* should be used for the actual “action” (*e.g.*, a heat treatment, an osmotic environment), while the injury may or may not result from the stress.

*Stress adaptation* is the “increase of an organism’s resistance to deleterious factors following exposure to mild stress” [(Yousef and Courtney 2003), p. 2]. Furthermore, “the phenomenon of one type of stress-response imparting auxiliary protection to cells subsequently stressed at higher levels” is known as *cross-protection* [(Wesche, Gurtler et al. 2009), p. 1125]. Stress response may “enhance resistance to subsequent processing conditions and/or enhance virulence” [(Chung, Bang et al. 2006), p. 52]. Cross-protection not only creates problems for the elimination of foodborne pathogens but also impacts microbial deterioration of food (Vorob'eva 2004).



Author's rendition of image from Yousef, A. E. and P. D. Courtney (2003). Basics of stress adaptation and implications in new-generation foods. Microbial Stress Adaptation and Food Safety. A. E. Yousef and V. K. Juneja. Boca Raton, FL, CRC Press: 1-30., p. 3.

**Figure 1.3: Interrelations among physiological states of microbial cells subjected to stress**

#### 1.3.2.2. Stress responses.

Upon sensing a stress, many different stress responses occur. This can include:

1. *Production of proteins that repair damage, maintain the cell, or eliminate the stress agent.*
2. *Transient increase in resistance or tolerance to deleterious factors.*
3. *Cell transformation to a dormant state (i.e., spore formation or passage to the viable-but-not-culturable state).*
4. *Evasion of host organism defenses.*
5. *Adaptive mutations.*

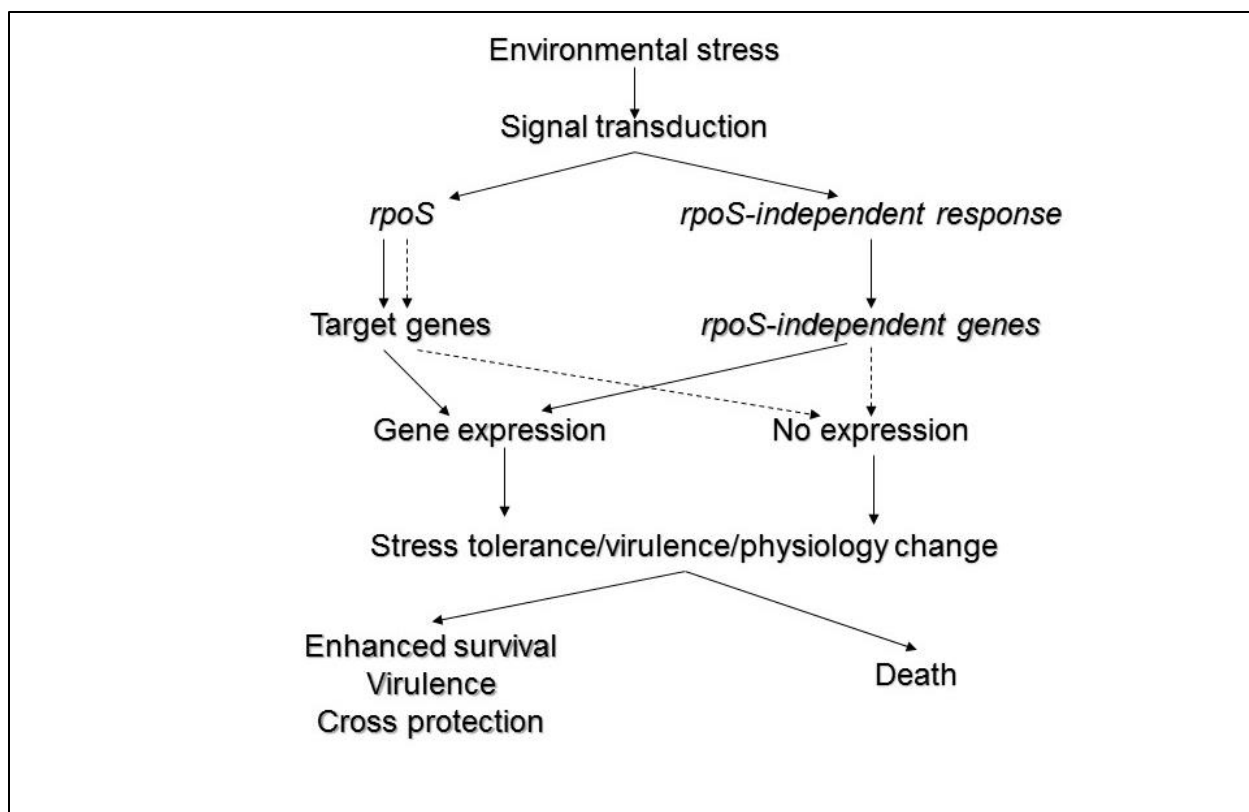
(Yousef and Courtney 2003), p. 4

Stressed organisms may enter an adaptive response in which tolerance (or, stress acclimatization) occurs. Some bacteria already possess a tolerance for certain stresses, while other bacteria require the adaptive response to achieve tolerance (Yousef and Courtney 2003).

Nevertheless, bacteria face a “dynamic environment” comprised of “potentially lethal threats” and “rapidly changing conditions” (Boor 2006, Ron 2006).

Stress response involves global regulatory networks that up- or downregulate various genes, known collectively as the stress response (Ron 2006). Much investigation has been undertaken to understand the general stress response of *E. coli*. Scientists posit (and in many cases have confirmed) that similar mechanisms apply to other Gram-negative microorganisms. *E. coli* uses signal transduction to respond to environmental stresses and activate genetic regulatory mechanisms, and these regulatory mechanisms likely evolved from a common prototype and are not unique to species or genus (Kennelly and Potts 1996, Chung, Bang et al. 2006). The main regulatory mechanism uses small proteins that bind to RNA polymerase known as *sigma factors* ( $\sigma$ ) (Chung, Bang et al. 2006). RNA polymerase is critical to cellular metabolism, recognizing chemical signals that denote a changing external environment and prompting the creating of mRNA transcripts to build new proteins (Boor 2006). The association of sigma factors with RNA polymerase results in the RNA polymerase holoenzyme “to recognize different promoter sequences and express entirely new sets of target genes” to build proteins that are better adapted to surviving particular stresses (Boor 2006).

The “master regulator” sigma factor, leading to the general stress response, is RpoS ( $\sigma^S$ ) (Small, Blankenhorn et al. 1994, Hengge-Aronis 1996). When induced by a stress (*e.g.*, weak acids, starvation, high osmolarity, temperature), RpoS transcriptionally causes regulatory changes in an estimated 500 genes that orchestrate morphological and physiological changes (Ron 2006). RpoS levels are regulated by transcriptional control, translational control, and post-translational control (Hengge-Aronis 1996, Dodd and Aldsworth 2002).



Author's abbreviation and rendition of diagram at Chung, H. J., et al. (2006). "Stress Response of *Escherichia coli*." *Comprehensive Reviews in Food Science and Food Safety* 5(3): 52-64., p. 53.

**Figure 1.4: Model for stress response of bacteria.**

RNAP—RNA polymerase. Solid line represents positive control.  
Dotted lines represent negative control.

While RpoS is involved in the general stress response, there are specific stress responses as well. These stress responses work alongside the master regulator during times of specific stress. Heat stress is mediated by the alternative sigma factor  $\sigma^{32}$  that induces heat shock proteins (HSPs) that act as chaperones to bind and stabilize nonnative polypeptides, prevent protein misfolding, and prevent protein aggregation (Georgopoulos and Welch 1993, Juneja, Klein et al. 1998, Chung, Bang et al. 2006, Wesche, Gurtler et al. 2009). HSPs may also assist in other cellular activities, as well, including morphological changes and DNA repair (Wesche, Gurtler et al. 2009). Two other sigma factors,  $\sigma^E$  and  $\sigma^{54}$ , are involved in heat stress response,

with  $\sigma^E$  assisting in outer membrane protein assembly and repair (the specific activity of  $\sigma^{54}$  is unknown) (Raivio and Silhavy 2001, Yousef and Courtney 2003).

When exposed to cold stress, *E. coli* modifies its cell membrane to maintain fluidity and structure (Chung, Bang et al. 2006). Cold shock proteins (CSPs) have been isolated from *E. coli*, *Salmonella*, and other foodborne pathogens, both Gram-positive and Gram-negative (Wesche, Gurtler et al. 2009). CSPs act as nucleic acid chaperones, overcoming inefficiencies in translation, transcription, and replication of DNA that occur when hydrogen bonds stabilize at cold temperatures (Juneja, Klein et al. 1998, Yousef and Courtney 2003, Wesche, Gurtler et al. 2009).

Acid stress, organic or inorganic, is a common stress encountered by bacteria. Reaction can involve several mechanisms, including “changes in membrane composition, increase in proton efflux, increase in amino acid catabolism, and induction of DNA repair enzymes” (Yousef and Courtney 2003). Many bacteria have specific acid tolerance or resistance systems, known as the acid tolerance response (ATR). The exposure to *moderately* low pH, and subsequent activation of an ATR, can result in protein synthesis that permits survival at *extremely* low pH (Yousef and Courtney 2003). In *E. coli*, there are three pathways for acid resistance: an oxidative system (ATR1), fermentative acid and resistance systems involving a glutamate decarboxylase (ATR2), and an arginine decarboxylase (Lin, Smith et al. 1996). When exposed to acidic conditions, *Salmonella* expresses between 48 and 60 acid shock proteins for protection (Audia, Webb et al. 2001, Wesche, Gurtler et al. 2009). Response to acid stress is often dependent on the growth phase of *E. coli*, with stationary phase in cells more tolerant than those in logarithmic phase (Buchanan and Doyle 1997).

Osmotic stress occurs when bacteria are subjected to high salt, high sugar, or dry conditions. In such an environment, water freely diffuses out of the cell. However, cells must maintain a positive turgor against the cell wall to survive (Csonka 1989, Yousef and Courtney 2003, Vorob'eva 2004). To achieve this goal, cells accumulate compatible solutes (sugars, polyols, free amino acids, quaternary amines, *et cetera*) to increase the cytoplasmic volume and free water content of the cell and to preferentially hydrate essential proteins (Yancey, Clark et al. 1982, Kempf and Bremer 1998).

In times of nutrient-limiting, or starvation, stress, *E. coli* will stop all growth and metabolism and begin production an array of enzymes (Chung, Bang et al. 2006). Some enzymes are degradative (*e.g.*, proteases and lipases) while others capture substrates (*e.g.*, glutamine synthetase and alkaline phosphatase) (Kjelleberg, Hermansson et al. 1987, Matin, Auger et al. 1989, Siegele and Kolter 1992, Chung, Bang et al. 2006). Starvation impacts cell shape and fatty acid composition and prompts storage of beneficial compounds in the cell (Chung, Bang et al. 2006). Some of the genes upregulated during starvation stress include *cst* (carbon stress) and *pex* (carbon, nitrogen, and/or phosphorous stress) which produce proteins that work alongside the general stress response ( $\sigma^S$ ) to aid in *E. coli* survival. Pathogens that are “successful” in surviving and multiplying in an environment do so by expressing virulence determinants, taking in signals from the external environment to up- or downregulate genes (Archer 1996).

## **1.4. ANTIMICROBIAL RESISTANCE**

### **1.4.1. Introduction, and a word on antibiotics versus antimicrobials.**

After years of use, many believe that humanity is “at the dawn of a postantibiotic era” [(Laxminarayan, Duse et al. 2013), p. 1057]. In such an era, human medicine will face a time when both commonplace and critical procedures (including major surgery and chemotherapy)

are no longer feasible because the likelihood of infection becomes too great. In fact, the medical community is already using “more expensive and more broad-spectrum antibiotics” as simpler antibiotics become ineffective [(Laxminarayan, Duse et al. 2013), p. 1059]. The United Nations has warned public health officials that the dumping of antibiotics, drugs, and chemicals into water and soil systems is allowing drug-resistant bacteria to “brew” in the natural environment (Le Roux 2017). Discharge from agricultural and industrial waste results in detectable antibiotic concentrations in waterways and soil (Le Roux 2017).

As microbes are exposed to antimicrobials, certain subsets of the population survive through genetic mutation, evading the mode of action of the antimicrobial and surviving. This *selective pressure* gives mutated strains the ability to carry in their genetic code antimicrobial resistance (AMR) characteristics, and that resistance is passed onto progeny (Davidson and Harrison 2002, Laxminarayan, Duse et al. 2013). While the use of antibiotics in human medicine has led to an increase in antimicrobial resistant organisms, emphasis has also been placed on the role of the food system, particularly within concentrated animal feeding operations (CAFOs) and food processing facilities. Food, like humans, has become international, and antimicrobial resistant bacteria originating in that food is shipped across oceans and continents every day in beef briskets, chicken breasts, and pork chops (Laxminarayan, Duse et al. 2013). Antimicrobials, particularly sanitizing agents, have been used in food manufacturing for over a century (Davidson and Harrison 2002). Yet, while scientists are well-versed in the mechanisms of action and resistance patterns that result in *antibiotic* resistance, little is known about the same characteristics for *antimicrobials* and *sanitizers* (Davidson and Harrison 2002).

In this section both *antimicrobial* and *antibiotic* resistance are explored. Antimicrobials are agents that destroy microorganisms, including antiseptics, disinfectants, sanitizers,

antibiotics, and antifungals. Thus, antibiotics are considered part of the antimicrobial family. In chapter 2, changes in sanitizer susceptibility. Lessons from antibiotic resistance and susceptibility are used in this literature review to better understand the potential for antimicrobial, *i.e.* sanitizer, resistance.

#### **1.4.2. AMR and the food system.**

Human medicine use of antibiotics is an important topic; however, for brevity and focus, only the impact of food production on resistance is explored in this literature review. Animal agriculture has used antibiotics at subtherapeutic levels since the 1940s; doing so kept animal disease at bay and brought animals to market quicker than traditional methods (Laxminarayan, Duse et al. 2013). As animal production shifted to a vertical enterprise, antibiotic use became the norm (Silbergeld, Graham et al. 2008).

One of the main ways AMR develops in the food system is at CAFOs. They are the ideal environment for AMR to develop and proliferate: tightly-packed animals live in dusty and dirty conditions where bacteria can proliferate and spread. In fact, Silbergeld et al. (2008) argue that the battle against AMR should begin in the animal agriculture arena for three reasons: “[1] It is the largest user of antimicrobials worldwide; [2] much of the use of antimicrobials in animal agriculture results in subtherapeutic exposures of bacteria; [3] drugs of every important class are utilized in agriculture; and [4] human populations are exposed to antimicrobial-resistant pathogens via consumption of animal products as well as through widespread release into the environment [p. 151].” Animal producers abroad often add heavy metals to feed which results in long-term co-selective pressure for resistance genes (Zhu, Johnson et al. 2013). For example, in a longitudinal study of *Salmonella* isolated from beef cows, a majority of isolates were resistant to two or more antimicrobials, and nearly half contained the integrase 1 gene which is significantly

associated with resistance to amoxicillin/clavulanic acid, chloramphenicol, ampicillin, streptomycin, sulfizoxazole, and tetracycline (Nesemeier, Ekiri et al. 2015).

AMR genes often accumulate in CAFO wastewater lagoons (Zhang, Snow et al. 2013, Brooks, Adeli et al. 2014). A survey of 37 swine farms identified genes conferring resistance to tetracycline and erythromycin among sow, nursery, and finisher farms (Brooks, Adeli et al. 2014). Analysis of storage ponds at Nebraska cattle feedlots discovered ABR genes located on the same bacterial genetic elements, purporting the theory that antimicrobials that select for one antibiotic-resistant gene may co-select for resistance to other antibiotics. Wastewater and runoff from CAFOs and animal harvesting facilities can lead to soil contamination or find its way into irrigation water for crops for human consumption (Silbergeld, Graham et al. 2008). Another study attained “proof of concept” that starlings—landing on, in, and around CAFOs—could promote the transfer of AMR *Salmonella* and AMR genes by flight and mechanical movement (Carlson, Hyatt et al. 2015). Researchers have identified “significant potential for widespread distribution of antibiotics, bacteria, and genetic material” by dust and airborne particulate matter, particularly downfield of cattle CAFOs [(McEachran, Blackwell et al. 2015), p. 341-342].

European Union (EU) nations started restricting the use of antibiotics as growth promoters in the 1970s, and a total ban was adopted in 2006 (Laxminarayan, Duse et al. 2013). The European Food Safety Authority (EFSA) has reported a correlation between lower sales of antimicrobials and lower antimicrobial resistance rates, overall (Laxminarayan, Duse et al. 2013). In the United States, the Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) is the primary authority on addressing antimicrobial resistance. The agency has “developed a multipronged strategy designed to limit or reverse resistance arising from the use of antibiotics in food-producing animals, while continuing to ensure the availability of safe and

effective antibiotics for use in animals and humans” (Food and Drug Administration 2017). This includes issuing guidance on judicious use, guidance on phasing out production uses, and limiting the use of certain cephalosporins and prohibiting the use of fluoroquinolones in poultry production (Food and Drug Administration 2017).

In 1996, a partnership was established between FDA, USDA, and CDC (Food and Drug Administration 2017). Known as the National Antimicrobial Resistance Monitoring Systems (NARMS), the surveillance system tracks antimicrobial resistance in enteric bacteria isolated from human, retail meat, and food animal sources (Centers for Disease Control and Prevention 2013, Centers for Disease Control and Prevention 2015). NARMS assists epidemiologists by identifying emerging resistance trends, increasing understanding about genetic spread of resistance, and promoting rulemaking about prudent antimicrobial usage (Centers for Disease Control and Prevention 2015). Proper funding for CDC, and in turn, NARMS, continues the critical effort of understating “the emergence, persistence, and spread of antibiotic resistance” and will hopefully give impetus for sensible stewardship of antibiotic use in humans and animals (Centers for Disease Control and Prevention 2013). In 2017, CDC awarded \$77 million for states to detect known and emerging antibiotic resistance threats in medicine and food (Anonymous 2017).

### **1.4.3. Antimicrobials and stress.**

Antimicrobials, particularly sanitizers, have been used by the food industry for years, with little evidence of developed resistance (Davidson and Harrison 2002). In their review, Davidson and Harrison (2002) state that concern about antimicrobial resistance remains because (1) “the increasing incidence of microorganisms exhibiting resistance to antibiotics used for therapeutic purposes in human and animal medicine” (which could signal AMR generally, not

solely ABR, occurring in the future), (2) “the increasing reliance on antimicrobials and sanitizers as primary tools for controlling the outgrowth of pathogens in foods”, and (3) “the evidence indicating that tolerance to antimicrobials, sanitizers, and other preservation processes may be generated within microorganisms exposed to certain stresses” [p. 69]. Furthermore, “food preservation regimes utilize environmental stresses such as pH extremes, temperature, and chemical preservatives to inhibit microbial pathogens and decomposers” (Zook, Busta et al. 2001) [p. 767].

#### **1.4.3.1 Examples of stressors and resulting susceptibility/resistance.**

**Acid stress.** Organic acids are efficient at reducing the population of *Salmonella* spp., *E. coli*, and other bacteria on beef tissue (Dickson and Siragusa 1994). Acid-adapted *Salmonella* Typhimurium demonstrated tolerance to other environmental stresses, an activated lactoperoxidase system, and surface-active chemical agents (Leyer and Johnson 1993). In another study, stationary phase acid tolerance response (ATR) of *Salmonella* was attempted by low pH acid shock (2h) and acid adaptation (over 18 h). ATR was achieved in acid adaptation cells and resulted in 4.2-4.8 log greater survival of *Salmonella* to acid challenge compared to nonadapted and acid shocked cells (Bacon, Sofos et al. 2003).

When exposed to sublethal concentrations of peroxyacetic acid (PAA) antimicrobial, *E. coli* O157:H7 displayed increased tolerance to peroxidative stress, but exposure to sublethal levels of acetic acid did not confer tolerance (Zook, Busta et al. 2001). The impact of acid adaptation on heat resistance of *L. monocytogenes* yielded mixed results based on strain and heating menstruum (Edelson-Mammel, Whiting et al. 2005).

**Alkaline stress.** Fats and proteins are often removed using alkaline cleaners (Sharma and Beuchat 2004). Alkaline cleaners are effective against Gram-negative bacteria because they

destroy the thin peptidoglycan layer (Sharma and Beuchat 2004). Experiments with *Salmonella* Enteritidis demonstrated that high pH (10-11) results in disrupted, permeable membranes (both outer and cytoplasmic) that release intracellular contents and result in cell death (Sampathkumar, Khachatourians et al. 2003).

Sharma and Beuchat (2004) exposed *E. coli* O157:H7 to seven commercial cleaners containing various concentrations of NaOH and KOH with pH values ranging from 10.41-11.71 and evaluated thermotolerance and antimicrobial resistance of survivors. While *E. coli* O157:H7 did not attain antimicrobial resistance after surviving alkaline cleaner challenge, some cells did show greater thermotolerance than control (Sharma and Beuchat 2004). *L. monocytogenes*, isolated from a food production facility, exhibited longer D-values (at 56 and 59°C) after growth in broth pH adjusted to 12.0 versus pH 7.3 (Taormina and Beuchat 2001). Pre-exposure to alkalization (growth in 9.0 pH-adjusted LB broth) resulted in *Vibrio parahaemolyticus* with increased resistance to heat, crystal violet, hydrogen peroxide, and deoxycholic acid, but not acid or alkaline stress (Koga, Katagiri et al. 2002).

**Freeze-thaw stress.** Freeze-thawing and rapid cooling has been shown to increase cell hydrophobicity in *Salmonella* and a resultant release of lipopolysaccharide (LPS) (Boziaris and Adams 2001). Such changes make the cell more susceptible to antimicrobials.

**Heat stress.** Heating, particularly that which is sublethal in nature, often results in “an increase of cell surface hydrophobicity, loss of components from outer and cytoplasmic membranes, inactivation of cell enzymes, degradation of rRNA and breaks in single DNA strands”, all of which can lead to mutation (Czechowicz, Santos et al. 1996). Similar to freeze-thaw, changes in cell hydrophobicity and LPS release have been noted during heating to 55°C (Boziaris and Adams 2001). A genomic island common to *Enterobacteriaceae*, including

*Salmonella enterica* and *E. coli*, confers greater heat resistance to the bacteria possessing the island, known as the locus of heat resistance (LHR) (Mercer, Walker et al. 2017). Further, by growing the LHR-positive cells in NaCl supplemented media (4%) improved survival of *S. enterica* and *E. coli* when heat challenged (Mercer, Walker et al. 2017). This resistance is likely due to accumulation of compatible solutes, which is discussed below. Exposing *E. coli* O157:H7 to sublethal levels of PAA did not result in heat resistance (Zook, Busta et al. 2001). In another study, heat shocking *E. coli* O157:H7 prior to further heat treatment resulted in more injured (and leaking) *E. coli* cells in the heat-shock group than control; however, the heat shocked cells had enhanced recovery ability during post-heat treatment anaerobic storage (Murano and Pierson 1993). Survival of *Salmonella* Typhimurium was enhanced when the cells were pre-incubated at 42°, 45°, or 48°C prior to heating at 55°C for 25 min, increasing the 7D inactivation time by a factor of 2.6 to 20-fold (Mackey and Derrick 1986). The increase in heat resistance phenomenon was also attained in liquid whole egg, reconstituted dried milk, and minced beef (Mackey and Derrick 1987). Heating has resulted in increased cellular sensitivity which can be overcome in the presence of oxygen scavenging materials (*e.g.*, pyruvate and catalase) and in a state of anaerobiosis (Czechowicz, Santos et al. 1996).

When *L. monocytogenes* was stressed by starvation or treatment with hydrogen peroxide, ethanol, and acidic pH, increases in thermotolerance resulted, with D-values of stressed cells significantly higher than control counterparts (Lou and Yousef 1996). Similar results were seen when *L. monocytogenes* was grown at varying temperatures and heat stressed, with the greatest increase in thermotolerance occurring when heat shock was more extreme (*e.g.*, cells grown at 4°C were shocked at 42.5 and 62°C) (Pagán, Condón et al. 1997). Similarly, *L. monocytogenes* inoculated in sausage and heat shocked for 120 min at 48°C prior to full heating to 62 or 64°C

(Farber and Brown 1990). Sublethally heat-shocked *Salmonella* also resulted in greater thermotolerance, increasing the D-value by 1-2 min at 57.8°C (Bunning, Crawford et al. 1990).

**Salt stress.** Salt addition “is a long established and widely applied means of extending the shelf-life of food products” (McMahon, McDowell et al. 2007), [p. 277]. Sublethal levels of salt, or osmotic, stress prompts *Salmonella* Virchow to change morphotype and better survive, with the capability of reverting back to normal morphology following the removal of the stress (McMahon, McDowell et al. 2007). High salt, low- $a_w$  conditions may prompt *Salmonella* to enter a filamentous state to survive until favorable conditions return (Mattick, Jørgensen et al. 2000).

**Cold.** At cold temperatures, *Salmonella* can form multicellular filamentous cells and revert to normal cell shape upon shifts to preferred (37°C) temperatures (Mattick, Phillips et al. 2003). Cold shocking *E. coli* induces the production of several genes, including those coding for the RNA chaperone CspA, the cold shock protein CspB, and CsdA, a helix destabilizing protein that assists in translation (Jones, Mitta et al. 1996). Furthermore, temperature depression increases the synthesis of ribosomal proteins that likely act as RNA chaperones (Jones, Mitta et al. 1996). Lower temperatures increased the efficacy (decreased the MIC) of methyl paraben against *L. monocytogenes*, *Pseudomonas putida*, and *Yersinia enterocolitica* (Moir and Eyles 1992).

**Other stressors and considerations.** Antimicrobial resistance may be passed by resistance plasmids gained during animal production, as seen in a survey of *L. monocytogenes* strains isolated from poultry carcasses. The strains exhibited resistance to benzalkonium chloride, hexamidine diisethionate, and ethidium bromide, and the resistance plasmid was transferred to non-resistant *L. monocytogenes* in conjugation experiments (Lemaitre,

Echchannaoui et al. 1998). In another study, exposure of *L. monocytogenes* to sublethal levels of antimicrobial (*i.e.*, an acidic anion antimicrobial, a chlorine-based antimicrobial, an iodophor, and a quaternary ammonium compound, as well as citric, lactic, and propionic acids) followed by exposure to lethal levels of the same antimicrobial generally did not confer any difference in survival (Pickett and Murano 1996). However, strains of *E. coli* resistant to household cleaners containing pine oil were also resistant to multiple antibiotics (*i.e.*, tetracycline, ampicillin, chloramphenicol, nalidixic acid), likely because both the pine oil and antibiotics are regulated by the same efflux pump; thus, the presence of an antimicrobial selected for chromosomal antibiotic resistance (Moken, McMurphy et al. 1997). In another study, the presence of ABR genes in *Salmonella* did not confer resistance to common beef processing antimicrobials (acetic acid, lactic acid, FreshFX), nor did the presence of said genes impact the efficacy of electrolyzed water, hot water, or ozone (Arthur, Kalchayanand et al. 2008). However, Brashears et al. (2001) showed that antibiotic-resistant strains of *E. coli* O157:H7 and *Salmonella* spp. were less sensitive to acidic conditions in an evaluation of general, recovery, and selective media. The use of nisin in concert with other sub-lethal levels of interventions (*i.e.*, heating, chilling, and freeze-thawing) enhanced nisin penetration and increased cell death (Bozianis and Adams 2001). Cold-challenged (8°C) *Salmonella* Enteritidis forms multicellular filaments as a survival mechanism. Upon transfer to 37°C, the filaments rapidly septate and form daughter cells at 150-fold per filament within 4 h (Mattick, Phillips et al. 2003). Subjecting *Salmonella* Typhimurium and *E. coli* O157:H7 to starvation stress decreased cellular ability to attach to beef tissue, and *Salmonella* was more sensitive to acetic acid following starvation (Dickson and Frank 1993). Repeat exposures to sodium hypochlorite, chlorhexidine digluconate, and benzalkonium chloride did not confer resistance to those antimicrobials, nor cross-resistance to other antimicrobials, for

*E. coli* O157:H7, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *L. monocytogenes* in both liquid media and associated with a surface (Riazi and Matthews 2011).

#### **1.4.4. The relationship of stress and hurdle technology.**

Many food production systems utilize hurdle technology to inhibit microbial growth and aid in preservation (Leistner and Gorris 1995, Montville and Matthews 2008). Hurdle technology involves preservation techniques (water activity, temperature, pH) used in concert that, taken together, are difficult for a microorganism to survive (Leistner and Gorris 1995). Figure 1.5 lists potential hurdles used in the preservation of foods. Hurdle technology “assaults multiple homeostatic processes”, and “when cells channel the energy needed for growth into maintenance of homeostasis, their growth is inhibited” (Montville and Matthews 2008) [p. 28-29].

**Physical hurdles:**

High temperature (sterilization, pasteurization, and blanching), low temperature (chilling and freezing), ultraviolet radiation, ionizing radiation, electromagnetic energy (microwave energy, radiofrequency energy, oscillating magnetic field pulses, and high electric field pulses), photodynamic inactivation, ultrahigh pressure, ultrasonication, packaging film (plastic, multi-layer, active coatings, and edible coatings), modified-atmosphere packaging (gas packaging, vacuum, moderate vacuum, and active packaging), aseptic packaging, and food microstructure.

**Physiochemical hurdles:**

Low water activity ( $a_w$ ), low pH, low redox potential (Eh), salt (NaCl), nitrite, nitrate, carbon dioxide, oxygen, ozone, organic acids, lactic acid, lactate, acetic acid, acetate, ascorbic acid, sulphite, smoking, phosphates, glucono- $\delta$ -lactone, phenols, chelators, surface treatment agents, ethanol, propylene glycol, Maillard reaction products, spices, herbs, lactoperoxidase, and lysozyme.

**Microbially derived hurdles:**

Competitive flora, protective cultures, bacteriocins, and antibiotics.

**Miscellaneous hurdles:**

Monolaurin, free fatty acids, chitosan, and chlorine.

Adapted from Leistner and Gorris (2005)

**Figure 1.5: Potential hurdles technology options for use in the preservation of foods.**

Often, antimicrobials (particularly sanitizers) are combined with one or several hurdles to inhibit bacterial growth (Davidson and Harrison 2002). Hurdle technology's main goal "is to inhibit the growth and proliferation of undesired organisms rather than to actually kill them, thus allowing for the use of hurdles that are not too extreme" (Leistner and Gorris 1995) [p. 45].

Hurdle technology and resistance characteristics are a Catch-22 of sorts: while using multiple hurdles to destroy pathogens can be effective at controlling spoilage microbes and pathogens, the same hurdle interventions may also select for stress-adapted, cross-protected organisms that will survive treatments and potentially cause illness. As Archer (1996) states: "Even though we must

continue to add stresses to foods, if those stresses are potentiating virulence or, worse, causing unpredictable adaptive genetic changes in virulence genes, there is a need to be aware of such occurrences” [p. 95].

#### **1.4.5. Biofilms, stress, and resistance.**

Biofilms are “a community of microorganisms attached to a surface, producing extracellular polymeric substances (EPC) and interaction with each other” [(Lindsay and Holy 2006), p. 27]. Specifically, biofilms are a matrix “of proteinaceous components including various adhesins as well as amyloids such as curli fibers, which can be interwoven with the exopolysaccharides”, including cellulose and colanic acid [(Mika and Hengge 2013), p. 4561]. Food processing “selects for biofilm forming bacteria... due to mass production of products, lengthy production cycles, and vast surface areas for biofilm development” [(Lindsay and Holy 2006), p. 31]. Not only can biofilms be difficult to clean and often are quite pervasive through a processing facility, they may be able to develop antimicrobial resistance due to their longevity, compared to sessile organisms (Lindsay and Holy 2006, Bae, Baek et al. 2012).

Thus, the control and eradication of biofilms in the food processing environment is critical to achieve food safety and prevent antimicrobial resistance. After growing *L. monocytogenes* on glass slides immersed in medium to form a biofilm, adherent cells showed more resistance to benzalkonium chloride, anionic acid antimicrobial, and heat compared to planktonic cells (Frank and Koffi 1990). Another study demonstrated that antimicrobial resistance in biofilms is dependent on the food material comprising the biofilm, the surface to which the biofilm is attached, and relative humidity of the system (Bae, Baek et al. 2012). Similarly, *Salmonella* Typhimurium biofilms showed an increased resistance to quaternary

ammonium compounds, mixed peroxyacetic acid/organic acid compounds, and sodium hypochlorite with age, temperature, and pH changes (Nguyen and Yuk 2013).

#### **1.4.6. Conclusion.**

AMR, including ABR and resistance to other antimicrobials, not only damages animal and human health, it also leads to decreases in “productivity and economy” in the food system [(Laxminarayan, Duse et al. 2013), p. 1070]. Exploration of new antibiotics and antimicrobials must continue to occur. For example, a new antibiotic named teixobactin was discovered in a screen of uncultured soil bacteria and, by virtue of binding to a highly conserved motif of lipid II and III (and not targeting a protein), does not develop resistance (Ling, Schneider et al. 2015). Nevertheless, food safety researchers must continue to understand the potential impacts food production may have on AMR and, through responsible stewardship, try and prevent or slow AMR from occurring.

### **1.5. MEAT SAFETY**

#### **1.5.1. Introduction to meat safety.**

Meat safety, similar to other areas of food safety, involves the elimination of hazards, be they microbiological, chemical, physical, or radiological. Meat safety issues that impact public health and/or require a recall, are often the target of interventions (Sofos 2008). Chief among the hazards associated with meat is the presence of pathogens, and typically this includes STEC, *Salmonella*, and *Campylobacter* (Sofos 2008). Pathogen elimination “should be based on an integrated approach which involves application of interventions at pre-harvest, post-harvest, processing, storage, distribution, merchandising, preparation, food service, and consumption” [(Sofos 2008), p. 8]. Often, contamination occurs during the slaughter process, and the occurrence is viewed as an unavoidable, but manageable event (Dickson and Anderson 1992).

Meat safety also encompasses non-microbial challenges, including: residues, additives, genetically modified organisms, bioterrorism, globalization of the food chain, animal welfare expectations, and sustainability initiatives (Sofos 2008).

### **1.5.2. The relationship to bacterial attachment.**

One way to mitigate the likelihood of pathogen presence in beef is to better understand the attachment of said pathogens on surfaces in food production facilities (including the product itself). In fact, the first step in bacterial contamination of meat products is attachment (Dickson and Anderson 1992). Often, this is viewed as a two-step process comprised of the primary reversible attachment and the irreversible, secondary attachment (Marshall, Stout et al. 1971). Yet, scientists know that the process is probably more complex, involving interactions at the molecular level that are not fully understood (Palmer, Flint et al. 2007). Factors impacting bacterial attachment are extrinsic or intrinsic in nature, and the influence of those factors on STEC attachment are used for illustration in this literature review.

Extrinsically, temperature is a major factor that determines attachment ability of STEC in beef (Tamplin, Paoli et al. 2005). Researchers have shown that *E. coli* O157:H7 can attach at temperatures associated with non-production conditions (*i.e.*, 15°C) and temperatures associated with cold storage (*i.e.*, 4°C) (Dourou, Beauchamp et al. 2011). STEC continue to attach during cold storage periods, and the rate of attachment increases as time shifts from hours to days (Dourou, Beauchamp et al. 2011). Other research demonstrates that the impact of temperature is mixed, at best. For example, growth temperature may impact attachment on adipose tissue but not lean tissue (Dickson 1991). Specifically, attachment to adipose tissue was greater at 23°C compared to 37°C. As temperature is increased, *Salmonella* and STEC cells grown in nutrient-limiting conditions had a decline in attached population, although not to a statistically significant

level (Dickson and Frank 1993). In another study, both temperature and tissue type did not demonstrate statistically significant differences in *E. coli* populations (Dickson and Siragusa 1994).

Another extrinsic factor is the growth media in which the bacteria is grown. In the natural world, the milieu can be a variety of condition, nutrients, and limiting factors. Attachment of *E. coli* to plant tissue is greatly impacted by growth media. The growth media causes downstream effects that change attachment capabilities, particularly capsule production (Hassan and Frank 2004). Cells grown in TSB (a nutrient dense-medium) and cattle manure extract (also a very favorable growth environment) were statistically similar (Cabedo, Sofos et al. 1997). Meat and poultry broths were also very hospitable for *E. coli* O157:H7 growth compared to other media (Silagyi, Kim et al. 2009). Conversely, cells grown in “starvation stress” media (which would be analogous to the hide, hooves, and general exterior of a cow) had a decreased bacterial attachment rate (Dickson and Frank 1993). Others agree that cellular stress induced by a minimal growth medium may influence cellular attachment (Patel, Sharma et al. 2011).

The final extrinsic factor is the meat surface itself. Consensus does not yet exist on the actual impact of lean or adipose tissue on attachment. Cabedo, Sofos et al. (1997) demonstrated no difference in STEC’s ability to attach to adipose or lean tissue. A thorough review of bacterial attachment by Frank (2001) determined that most research indicates that bacteria attach equally to lean and adipose tissue, and any variation that occurs is within 0.5 log<sub>10</sub> values.

Intrinsic factors also influence bacterial attachment (Goulter, Gentle et al. 2009, McWilliams and Torres 2014). Intrinsic factors include cellular appendages and surface proteins, such as the capsule, fimbriae, outer membrane polymers, S layers, and other attachment organelles (Frank 2001, Goulter, Gentle et al. 2009, McWilliams and Torres 2014). When

bacteria approach and come in contact with a surface, attachment organelles are synthesized (Lacanna, Bigosch et al. 2016). This is enhanced by the behavior of fimbriae and flagella (Rivas, Fegan et al. 2007, Patel, Sharma et al. 2011). Interestingly, STEC that can produce curli fimbriae are better at attaching than cells that cannot (Patel, Sharma et al. 2011, Carter, Louie et al. 2016). As better understanding about the mechanics of bacterial attachment is progressing, so are genetic insights: researchers have discovered an enzyme, diguanylate cyclase (DgcZ), that stimulates biosynthesis of exopolysaccharide PGA and interacts with the fumarate reductase complex to enhance STEC surface attachment (Lacanna, Bigosch et al. 2016).

While organelles and structures play a key role in attachment, so does the bacterial surface charge and hydrophobicity (Dickson and Koohmaraie 1989). The attraction between lean tissue and bacteria is influenced by negative charges on the bacterial cell surface (Dickson and Koohmaraie 1989). Conversely, attraction to adipose tissue is believed to be a function of hydrophobicity (Pringle and Fletcher 1983, Dickson and Koohmaraie 1989).

### **1.5.3. Control of pathogens: Antimicrobials, interventions, and HACCP.**

The use of antimicrobials in food, generally, has been discussed previously in this literature review. Specifically for meat, various types and classifications of antimicrobials have been effectively used by industry, including sodium chlorite, PAA, lactic acid, acetic acid, lauric arginate, cetylpyridinium chloride, sodium metasilicate, bromine, sulfuric acid plus sodium sulfate, chlorine, sodium hydroxide, various other organic acids, and combinations of the aforementioned (Dickson and Anderson 1992, Koohmaraie, Arthur et al. 2005, Geornaras, Yang et al. 2012, Kalchayanand, Arthur et al. 2012, Stella, Luchansky et al. 2017). Of course, the use of antimicrobials constitutes but one step in the process of eliminating bacteria from meat. As stated in Unruh, Kastner et al. (2016), and referencing work by Nutsch (1998) and Fung,

Hajmeer et al. (2001), such interventions “include proper handling of carcasses (*e.g.*, minimizing contact of the hide with the skinned carcass, performing environmental sanitation, and ensuring rapid carcass chilling), water washing, hot water washing,...trimming to remove filth and contaminants, steam pasteurization, and steam vacuuming” [p. 115]. USDA has established several requirements to ensure the safety of certain meat products, including Hazard Analysis and Critical Control Points (HACCP) systems, performance standards related to lethality and stabilization of certain ready-to-eat products, and time-temperature combination for partially cooked or premarked products (Unruh, Kastner et al. 2016).

## **1.6. PRODUCE SAFETY**

### **1.6.1. Introduction to produce safety.**

The conveyance of foodborne illness by fresh produce was a problem in the early 1900s, when cholera and typhoid fever were rampant (Tauxe, Kruse et al. 1997). In modern times, human pathogens (*e.g.* Hepatitis A and *Shigella*) and pathogens of animal reservoir are of concern (Tauxe, Kruse et al. 1997) Foodborne illness caused by produce is a major public health concern, with norovirus and *Salmonella* spp. causing the most illness in the United States during 2004-2012 (Callejon, Rodriguez-Naranjo et al. 2015). Multistate produce outbreaks in the United States are often attributed to *Salmonella* (Callejon, Rodriguez-Naranjo et al. 2015). *Salmonella* Newport and *Salmonella* Typhimurium are most commonly implicated in the U.S. (Callejon, Rodriguez-Naranjo et al. 2015). *E. coli* and *Campylobacter* spp. are commonly implicated in U.S. outbreaks, as well (Callejon, Rodriguez-Naranjo et al. 2015). The prevalence of foodborne illness outbreaks attributable to fresh produce “challenges the notion that enteric pathogens are defined mostly by their ability to colonize the intestinal habitat” [(Brandl 2006) p. 367]. It is estimated that 400 produce-related outbreaks have occurred since 1990 (Murray, Wu et al. 2017).

The increase in produce-linked outbreaks can be attributed to a wide variety of factors, which are summarized in Table 1-5. Sources of produce contamination include manure application, feces from domestic or wild animals, unhygienic field workers, contaminated water used for irrigation or pesticide application, bioaerosols, and/or insect transmission (Brandl 2006, Berger, Sodha et al. 2010).

**Table 1-5: Factors involved in the emergence of produce-linked outbreaks**

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|------------------------------------------------------------------------------|
| Changes in the produce industry                                              |
| Intensification and centralization of production                             |
| Wider distribution of produce over longer distances                          |
| Introduction of minimally processed produce                                  |
| Increased importation of fresh produce                                       |
| Changes in consumer habits                                                   |
| Increased consumption of meals outside the home                              |
| Increased popularity of salad bars                                           |
| Increased consumption of fresh fruits and vegetables, and fresh fruit juices |
| Increased size of at-risk population (elderly, immunocompromised)            |
| Enhanced epidemiological surveillance                                        |
| Improved methods to identify and track pathogens                             |
| Emerging pathogens with low infectious dose                                  |

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Adapted from Brandl (2006) as derived from Tauxe, Kruse et al. (1997).

A wide variety of products have been vehicles for foodborne illness outbreaks, including raspberries, strawberries, and green onions in hepatitis A outbreaks; raspberries in multiple *Cyclospora* outbreaks; tomatoes, melons, peppers, sprouted seeds, and leafy vegetables for *Salmonella* spp.; and leafy green vegetables and sprouted seeds in STEC outbreaks (Berger, Sodha et al. 2010). In a review on produce safety technologies, Murray, Wu et al. (2017) comment on the wide diversity of products being implicated in outbreaks, including the commonly implicated (*e.g.*, leafy greens, tomatoes, cantaloupes, and soft fruits) as well as the unique (*e.g.*, cucumbers and papaya) (Murray, Wu et al. 2017).

Research conducted on the common weed *Arabidopsis thaliana* has elucidated the behavior of plant immune responses when *Salmonella* is present. Chiefly, *Salmonella* can evade the plant host immune response, enter the cytoplasm, begin reproducing, and cause wilting, chlorosis, and plant death. The work with *A. thaliana* gives insight to the behavior of *Salmonella* in spinach and other leafy greens and can serve as a model for future produce safety interventions (Schikora, Carreri et al. 2008).

In 2006, a multistate outbreak of *E. coli* O157:H7 occurred, causing 225 cases (191 confirmed) across 24 states (Sharapov, Wendel et al. 2016). The cases were traced back to raw, fresh spinach sold in bags. Of those infected, 17% developed HUS and 2% died. The hospitalization rate was 55% (Sharapov, Wendel et al. 2016). Extrapolating for unreported cases, likely more than 5,000 infections resulted from this outbreak (Scallan, Hoekstra et al. 2011, Sharapov, Wendel et al. 2016). The HUS and death rate associated with this outbreak was more pronounced than in other STEC outbreaks. According to Sharapov, Wendel et al. (2016), this is probably attributable to three factors: (1) the strain only produced Stx2, which is associated with higher HUS rates; (2) the titer of bacteria in the bagged spinach was uncommonly high; and (3) spinach is often consumed raw, eliminating any kill step that may occur from heating (Sharapov, Wendel et al. 2016). The outbreak was notable not only for its scope, product-pathogen combination, and disease severity, it also highlighted the power of *one bad day*: evidence “confirmed that fresh produce produced by one firm, at one facility, on 1 day, and distributed nationally was the vehicle for pathogen transmission” [(Sharapov, Wendel et al. 2016) p. 2028].

Although there may be an increased risk for foodborne illness when consuming fresh fruits and vegetables, the nutritional benefit of consuming fresh produce outweighs the risk of becoming sick (Nyachuba 2010). Compared to pathogens, many consumers view pesticides as a

greater risk when consuming fresh produce, and many underestimate the probability of developing a foodborne illness from consuming fresh fruits and vegetables (Yu, Neal et al. 2018). Nevertheless, a majority of consumers would be willing to pay a \$1 premium for produce that is half-as-likely to cause them to be ill (Yu, Neal et al. 2018).

### **1.6.2. Antimicrobial interventions.**

Antimicrobial interventions have included L-lactic acid, peroxyacetic acid, calcium hypochlorite, ozonated water, ClO<sub>2</sub> gas, hydrogen peroxide, malic acid, among others. (Gil, Selma et al. 2009, Neal, Marquez-Gonzalez et al. 2012, Almasoud, Hettiarachchy et al. 2015, Zhang, Oh et al. 2015, Murray, Wu et al. 2017). Controlling microbes in produce is best achieved by combining antimicrobials with physical treatments; however, particular care must be given to ensure wash water does not shift from being an intervention to a contamination source (Gil, Selma et al. 2009).

Chlorine has been frequently used by the produce industry in water washes; however, the increasing numbers of outbreaks have challenged its efficacy (Ölmez and Kretzschmar 2009). Further, the environmental and health risks of chlorine have prompted producers to look elsewhere (Gil, Selma et al. 2009, Ölmez and Kretzschmar 2009).

Application of 2% L-lactic acid at 55°C resulted in a 2.7 log CFU/g reduction of *E. coli* O157:H7 and a 2.3 log CFU/g reduction of *Salmonella* spp. on spinach, significantly more reduction than water control. Application of 8.0 log<sub>10</sub> CFU/g lactic acid bacteria to *Salmonella*-inoculated spinach resulted in a reduction of 1.9 log<sub>10</sub> CFU/g *Salmonella*, with the reduction narrowing to 0.7 log<sub>10</sub> CFU/g at 12 d at 7°C compared to control (Cálix-Lara, Rajendran et al. 2014). Similar results were obtained for *E. coli* O157:H7, with an initial reduction of 1.6 log<sub>10</sub>

CFU/g that narrowed to 0.3 log<sub>10</sub> CFU/g at the end of storage (Cálix-Lara, Rajendran et al. 2014).

Using multiple, or combined, sanitization procedures has been effective as well. After inoculating spinach with *S. Typhimurium*, Zhang, Oh et al. (2015) subjected the product to a nano-aerosolized water dip, an H<sub>2</sub>O<sub>2</sub> dip, or a combination of both, and the number of surviving bacteria was 5.2, 4.7, and 1.1 CFU/g for each treatment, respectively. *E. coli*-inoculated baby spinach, sprayed with Pro-San L (0.66% citric acid and 0.036% sodium lauryl sulfate) followed by vacuum cooling and ozonation under pressure resulted in a 3.9 log<sub>10</sub> CFU/g reduction in microbial population (Pyatkovskyy, Shynkaryk et al. 2017). A combination of lactic acid and malic acid applied by electrostatic spraying was effective at reducing populations of *S. Typhimurium* and *E. coli* O157:H7 and also disrupted associated biofilms (Almasoud, Hettiarachchy et al. 2015).

While many have advocated for reduced product contamination of produce in the field and prevention of cross-contamination in processing and handling, continued outbreaks have prompted the need for other interventions aside from chemicals (Murray, Wu et al. 2017). Alternatives to chemical interventions include irradiation, ultraviolet light, high pressure processing, gas-phase treatments, advanced oxidative processes (the generation of hydroxyl radicals from ozone or hydrogen peroxide), and gas plasma (Murray, Wu et al. 2017). The transition of these techniques from the laboratory to industry presents both opportunities and challenges.

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## **Chapter 2 - Susceptibility of Shiga Toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. to Common Antimicrobials Following Stress**

### **2.1. ABSTRACT**

Using multiple stressors (usually physical or chemical) during food processing is a common technique to control pathogens. Ascertaining whether stressors commonly encountered by *Salmonella* and/or Shiga toxin-producing *Escherichia coli* (STEC) during processing impact antimicrobial efficacy can assist producers and regulators in determining chemical concentrations for judicious use. The purpose of this study was to determine if subjecting *Salmonella* and STEC cells to common slaughter/fabrication stressors prior to antimicrobial exposure impacts the minimum inhibitory concentration (MIC) of said antimicrobials. *Salmonella* Montevideo, Newport, and Typhimurium, and STEC O26, O45, O103, O111, O145, and O157:H7 were independently grown in tryptic soy broth (TSB) for 18 h at 37°C and exposed to one of six stresses: no stress (control), salt, acid, heat, freeze-thaw, and alkaline. Cultures were centrifuged and pelletized cells were adjusted to a 0.5 McFarland standard prior to inoculating Mueller-Hinton (MH) broth and dosing into a 96-well microplate containing various concentrations of the antimicrobials lauric arginate (LauArg), citric acid plus hydrochloric acid (CA+HCl), peroxyacetic acid plus acetic acid and hydrogen peroxide (PAA+AA), lactic acid plus citric acid (LA+CA), and lactic acid (LA). MICs were determined from absorbance value interpretations following 18 h growth at 37°C. For all serotype and antimicrobial combinations investigated, concentration of antimicrobial was significant ( $p \leq 0.05$ ). Stress was significant for *S. Montevideo* subjected to LauArg, CA+HCl, and PAA+AA ( $p \leq 0.05$ ). In regards to *S. Newport*, stress was significant when subjected to LA+CA, while concentration  $\times$  stress was significant

when exposed to CA+HCl, PAA+AA, and LA ( $p \leq 0.05$ ). Pertaining to *S. Typhimurium*, stress was significant for cells subjected to LA+CA, while concentration  $\times$  stress was significant for CA+HCl, PAA+AA, and LA ( $p \leq 0.05$ ). For STEC, concentration was significant ( $p \leq 0.05$ ) for all antimicrobials used versus all serogroups. Use of common processing stressors in concert with antimicrobials may increase or decrease the antimicrobial concentration required to destroy *Salmonella* and/or STEC. Continued monitoring of changes in efficacy related to processing stressors is warranted as ecological changes in microbial populations continue to occur and bacteria continue to evolve.

## 2.2. INTRODUCTION

The Centers for Disease Control and Prevention (CDC) estimates that *Escherichia coli* O157:H7 causes 96,000 illnesses annually, while an additional 168,000 annual illnesses are caused by the non-O157 *E. coli* serogroups O26, O45, O103, O111, O121, and O145 (known as the “Big Six”) (Scallan, Hoekstra et al. 2011). Furthermore, an estimated 93.8 million cases of nontyphoidal *Salmonella* occur each year, with 155,000 succumbing to the illness (Majowicz, Musto et al. 2010, Callejon, Rodriguez-Naranjo et al. 2015). STEC are associated with beef cattle and are often present at the time of processing (Ferens and Hovde 2011). Multistate produce outbreaks in the United States are often attributed to *Salmonella* (Callejon, Rodriguez-Naranjo et al. 2015), and *Salmonella* infections have been traced back to tomatoes, melons, peppers, and sprouted seeds (Berger, Sodha et al. 2010). *Salmonella* also is a persistent problem in beef and chicken, with high prevalence in both products (Fegan, Vanderlinde et al. 2004, Park, Aydin et al. 2014).

Antimicrobials have been used for over a century, and little evidence for antimicrobial resistance has been documented; nevertheless, the antibiotic-resistance crisis and overt

dependence on chemical antimicrobials makes the question of resistance-development absolutely relevant (Davidson and Harrison 2002). Changes in the resistance of a microorganism to an antimicrobial has been correlated with concurrent or consecutive stressors (Davidson and Harrison 2002). The use of antimicrobials is a common intervention in United States Department of Agriculture (USDA)-regulated product processing, as elaborated in FSIS Directive 7120.1 (USDA Food Safety and Inspection Service 2018). Knowledge gaps exist regarding antimicrobial mechanisms and their behavior in concert with other antimicrobials and/or hurdles (Davidson and Harrison 2002).

*Stress adaptation* is the “increase of an organism’s resistance to deleterious factors following exposure to mild stress” (Yousef and Courtney 2003). Furthermore, “the phenomenon of one type of stress-response imparting auxiliary protection to cells subsequently stressed at higher levels” is known as *cross-protection* (Wesche, Gurtler et al. 2009). Stress response may “enhance resistance to subsequent processing conditions and/or enhance virulence” (Chung, Bang et al. 2006).

Generally, experiments have demonstrated that repeat exposure to antimicrobials does not confer resistance. For example, repeat exposures to sodium hypochlorite, chlorhexidine digluconate, and benzalkonium chloride did not confer resistance to those antimicrobials, nor cross-resistance to other antimicrobials, for *E. coli* O157:H7, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Listeria monocytogenes* in both liquid media and associated with a surface (Riazi and Matthews 2011).

Therefore, the objective of this study was to determine if subjecting STEC or *Salmonella* spp. to commonly-encountered stressors (*e.g.*, acid, alkaline, freeze-thaw, heat, and salt) changed

the susceptibility of the microorganisms to various antimicrobials commonly used in the meat, produce, and other food processing industries.

## 2.3. MATERIALS AND METHODS

**Culture preparation and stress application.** Frozen stock cultures of five to six *Escherichia coli* O26, O45, O103, O111, O145, and O157:H7 (USDA-ARS) strains, and frozen stock cultures of single *Salmonella enterica* subsp. Montevideo (isolated from cattle), Newport (isolated from cattle), and Typhimurium (ATCC 14028) strains, stored in tryptic soy broth (TSB; BD Difco, Franklin Lakes, NJ) at -80°C, were revived from frozen and independently grown at 37°C for 18 h. Individual serogroups/subspecies were then subjected to one of the following stresses: control (transfer to TSB for 18 h growth at 37°C), acid (transfer to TSB for 16 h growth, pH adjusted to ~4.5 by addition of 1 N HCl for 2 h, all at 37°C), alkaline (transfer to TSB for 17.5 h growth, pH adjusted to ~11 by addition of 1 N NaOH for 0.5 h, all at 37°C), freeze-thaw (transfer to TSB for 15.5 h at 37°C, transfer to -20°C for 2 h, and thawed at room temperature for 0.5 h), and salt (transfer to TSB + 4% NaCl for 18 h growth at 37°C). Heat parameters were validated prior to experimentation while others were based upon the work of McMahon, Xu et al. (2007), in order to stress the cells without substantially decreasing populations. Subsequent to stress challenge, broth cultures of individual strains or serogroups were centrifuged at  $5,200 \times g$  for 15 min at 4°C (Allegra X-30R, Beckman Coulter, Brea, CA). The supernatant was discarded and the pellet was used for dosing.

**Antimicrobial preparation.** Stock solutions of antimicrobials were diluted in sterile, ddH<sub>2</sub>O. “LaurArg” had an active ingredient of 20% ethyl-*N*-dodecanoyl-L-arginate hydrochloride, or lauric arginate (Mirenat-GA; Vedeqsa Inc., Independence, MO). The active ingredients in “CA+HCl” were citric acid and hydrochloric acid (Citrilow™; Safe Foods

Corporation, North Little Rock, AR). “PAA+AA” was comprised of 50% acetic acid, 15% peroxyacetic acid (PAA), and 4.9% hydrogen peroxide (Birkoside MP-2™; Birko Corporation; Henderson, CO). Antimicrobial “LA+CA” contained lactic acid (43-49%) and citric acid (39-45%) (Beefxide™; Birko Corporation). Finally, antimicrobial “LA” was 88% lactic acid (Birko Corporation). The challenge ranges (concentrations) of antimicrobials performed in this study are given in Table 2.1.

**Dosing and microplate preparation.** Ninety-six well microplates (Costar 3370; Corning Inc., Kennebunk, ME) were dosed (100 µL) with antimicrobials at increasing concentrations across rows (Figure 2-1; Table 2.1). Resultant microbial pellets from aforementioned centrifugation were used to obtain a 0.5 McFarland standard in sterilized water (Thermo Fisher Scientific, Waltham, MA) using a nephelometer (Thermo Fisher Scientific, Waltham, MA). A 60 µL aliquot of standardized culture was transferred into 11 mL Mueller-Hinton broth (MH; Thermo Fisher Scientific, Waltham, MA), vortexed, and 150 µL of culture was dosed into microplates. Microplates were sealed with a protective film (Thermo Fisher Scientific, Waltham, MA), covered with lid, and incubated statically for  $24 \pm 2$  h at 37°C.

**Absorbance analysis.** Following 24 h incubation, film was carefully removed from microplates. Lids were returned to plates, and plates were inserted into a plate reader (Epoch, BioTek, Winooski, VT). Absorbance was read at 450 and 595 nm for *Salmonella* and STEC (Sutton 2006, Quigley 2008), respectively. For each antimicrobial concentration, two wells were analyzed, and background interference from the antimicrobial and MH was subtracted before combining to form a mean (Figure 2.1). Two growth controls were also performed for each bacteria-stress combination. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $<$

0.200 and  $> 0.100$  were considered “intermediate”, and readings  $\leq 0.100$  were considered “susceptible” (McMahon, Xu et al., 2007).

**Statistical analysis.** Experiments were performed in three replicates. Statistical analysis using PROC MIXED (Statistical Analysis Software 9.4, Cary, NC) was performed at the serogroup and antimicrobial level for antimicrobial concentration and stress, utilizing absorbance values. LS MEANS of the absorbance values were calculated to determine the MIC value for each stress and antimicrobial combination. MICs were determined when LS MEAN absorbance values were  $\leq 0.100$  at the lowest concentration.

## 2.4. RESULTS

**Impact of stress on *Salmonella* susceptibilities.** The individual (rep) results for *Salmonella* serogroups are shown in Appendix A. For all serotype and antimicrobial combinations investigated, concentration of antimicrobial was significant ( $p < 0.0001$ ). Stress was significant for *S. Montevideo* (Figure A1) subjected to “LaurArg” (active ingredient of 20% ethyl-*N*-dodecanoyl-L-arginate hydrochloride), CA+HCl (active ingredients citric acid and hydrochloric acid), and PAA+AA (comprised of 50% acetic acid, 15% peroxyacetic acid, and 4.9% hydrogen peroxide) ( $p \leq 0.05$ ). Meanwhile, only concentration was significant ( $p \leq 0.05$ ) for *S. Montevideo* subjected to LA+CA and LA.

In regards to *S. Newport* (Figure A2), stress was significant when subjected to LA+CA (containing lactic acid [43-49%] and citric acid [39-45%]) ( $p \leq 0.05$ ). The interaction of concentration  $\times$  stress was significant for *S. Newport* exposed to antimicrobials CA+HCl, PAA+AA, and LA (88% lactic acid ( $p \leq 0.05$ )). Finally, stress was significant ( $p \leq 0.05$ ) for *S. Typhimurium* (Figure A3) subjected to LA+CA, while concentration by stress was significant for CA+ HCl, PAA+AA, and LA ( $p \leq 0.05$ ).

**Impact of stress on *Salmonella* MIC designations.** For *S. Montevideo*, reductions in MIC were observed when cells were exposed to PAA+AA. Furthermore, all cells, except those heat stressed, were less susceptible to LaurArg (Table 2-2). However, stressed *S. Newport* and *S. Typhimurium* resulted in MICs that were the same as or less than the control, except *S. Newport* stressed by alkaline and subjected to LaurArg (Tables 2-3 and 2-4).

**Impact of stress on STEC susceptibilities.** Similar results were obtained for STEC strains. The individual (rep) results for STEC serogroups are shown in Appendix A. Concentration was significant ( $p \leq 0.05$ ) for all antimicrobials used versus all serogroups. For serogroup O26 (Fig A4), there was no difference ( $p > 0.05$ ) in stress or stress  $\times$  concentration for all antimicrobials. For serogroup O45, there was no significance ( $p > 0.05$ ) in stress or stress  $\times$  concentration for LaurArg and LA. For O45 subjected to CA+HCl, stress was significant ( $p \leq 0.05$ ) with differences between heat and all other stressors, particularly control. When O45 was subjected to PAA+AA, stress was significant ( $p \leq 0.05$ ). Specific differences were observed between heat and acid, heat and salt, alkaline and freeze-thaw, and most importantly, heat and control (more growth in control versus heat). Furthermore, stress  $\times$  concentration was approaching significance in the O45 experimentation ( $p = 0.0538$ ). Finally, for O45 subjected to LA+CA, stress was significant ( $p \leq 0.05$ ), with more growth occurring in acid than control.

For serogroup O103 (Figure A6), there was no significance ( $p > 0.05$ ) in stress or stress  $\times$  concentration for CA+HCl, LA+CA, or LA. Stress was significant ( $p \leq 0.05$ ) for LaurArg and PAA+AA. Specifically, for LaurArg, freeze-thaw grew more than control. For serogroup O111 (Figure A7), there was no significance ( $p > 0.05$ ) in stress or stress  $\times$  concentration for CA+HCl. Stress was significant for O111 and LaurArg ( $p \leq 0.05$ ), with acid and freeze-thaw outgrowing control. When antimicrobial PAA+AA was used to challenge O111, acid grew more than

( $p \leq 0.05$ ) control. For O111 and LA+CA, control was significantly different ( $p \leq 0.05$ ) from acid, alkaline, and salt.

When analyzing serogroup O145 (Figure A8), there was no significance ( $p > 0.05$ ) in stress or stress  $\times$  concentration for CA+HCl and LA+CA. For O145 challenged with LaurArg, acid and control were significantly different ( $p \leq 0.05$ ). Stress was also significant when O145 was challenged with PAA+AA ( $p \leq 0.05$ ). Specifically, salt and heat grew less than control. For O145 challenged with LA, stress was significant ( $p \leq 0.05$ ) and stress  $\times$  concentration was approaching significance ( $p = 0.0759$ ). Namely, the acid, freeze-thaw, and heat all differed from control. Finally, STEC O157:H7 demonstrated no significance ( $p > 0.05$ ) in stress or stress  $\times$  concentration for all antimicrobials.

**Impact of stress on STEC MIC designations.** STEC MICs demonstrated greater variability than those of *Salmonella*. MICs remained relatively constant when STEC was stressed and subjected to LA+CA and LA. When STEC was subjected to LaurArg, for example, MIC values of controls and stressors ranged from 22.5 to 40 ppm with no discernable pattern. For STEC subjected to CA+HCl, growth inhibition ranged from 1.50% to  $> 2.50\%$  concentration. When STEC cells were stressed and subjected to PAA+AA, MICs ranged from as low as 100 ppm to as high as 140 ppm, with varying increases and decreases in MIC compared to control. STEC O26 (Table 2-5) had MICs higher for the stressors than the control when challenged to LaurArg. The control MIC for STEC O45 (Table 2-6) challenged by CA+HCl was 1.75%, while all other MICs were higher, except heat. Similarly, STEC O103 (Table 2-7), along with STEC O145 (Table 2-9) were less susceptible than others to CA+HCl. Control MIC concentration for STEC O111 (Table 2-8) subjected to PAA+AA was 100 ppm; all others (except heat) were 110

ppm or greater. STEC O157 (Table 2-10) cells were consistent: control and stressors were the same MIC concentration for LaurArg, LA+CA, and LA.

## 2.5. DISCUSSION

Two approaches for discerning the susceptibilities of *Salmonella* and STEC were utilized in this study. First, a statistical analysis of absorbance values, which generated LSMEANS absorbance values, was employed to determine impacts of various stressors and antimicrobial concentrations. Then, LSMEANS absorbance values were used to determine MICs for each combination of stress and serotype/serogroup, which are generally discussed in terms of an increase or decrease in pathogen susceptibility to antimicrobials following various stressors.

Current scientific evidence demonstrates that little to no support exists for the development of microorganisms that are less susceptible or resistant to common antimicrobials or sanitizers (Davidson and Harrison 2002). Similar results were obtained in this study.

In this study, HCl was used to discern whether a non-organic acid could impact susceptibility, as was done by McMahon, Xu et al. (2007). Changes related to acid resistance are possibly a result of acid adaptation, which can result in resistance to other stressors and some chemicals (Leyer and Johnson 1993, Bacon, Sofos et al. 2003). Therefore, when acid-challenged cells did differ in susceptibility to antimicrobials compared to control in this study (albeit mildly), it may have been due to mild acid adaptation. However, exploring this mechanism specifically was beyond the scope of this study. In most cases, exposure to sublethal levels of acid did decrease susceptibility to antimicrobials, as others have demonstrated (Zook, Busta et al. 2001).

In previous research, *E. coli* O157:H7 exposed to alkaline cleaners did not demonstrate increased resistance to other antimicrobials, including cetylpyridinium chloride and

benzalkonium chloride, but did show increased heat resistance (Sharma and Beuchat 2004). This is similar to the results in this study: exposure to alkaline conditions prior to antimicrobial treatment did not impact efficacy of the antimicrobials. Contrary to what is reported here, other studies have shown that bacteria exposed to stress may become resistant to heat or other chemicals. For example, *L. monocytogenes* grown in alkaline conditions was more resistant to heat (Taormina and Beuchat 2001, Koga, Katagiri et al. 2002).

Freeze-thawing and rapid cooling has been shown to increase cell hydrophobicity in Gram negative bacteria, increasing susceptibility to antimicrobials (Bozianis and Adams 2001). Occasionally, freeze-thaw resulted in a decrease in MIC compared to control (particularly when challenged with PAA+AA, as was the case for *S. Montevideo*, *S. Typhimurium*, and STEC O45). On the other hand, freeze-thaw resulted in an increase in MIC compared to control in some instances (for example, *S. Montevideo*, STEC O45, and STEC O111 all had CA+HCl MICs higher than control). Generally, freeze-thaw did not provide bacteria a substantial survival advantage, as increases in MIC were relatively small.

Perhaps the most widely-studied aspect of bacterial inactivation is that achieved by heat. Sublethal heat can cause cell hydrophobicity, loss of cellular components from membranes, and changes in DNA that can result in mutations favorable for survival (Czechowicz, Santos et al. 1996). In this study, exposure to sublethal heat generally resulted in a lower MIC for the antimicrobials evaluated in comparison to control. Specifically, in the forty-five iterations of heat stress  $\times$  antimicrobial  $\times$  bacteria, heat increased the susceptibility (lowered the MIC) twenty-seven times, and only increased the MIC once (LaurArg O26). In another study, initial heat shocking of STEC allowed cells to more readily withstand subsequent chemical and heat challenge (Murano and Pierson 1993). Such a result, where exposure to heat resulted in enhanced

survival in the presence of antimicrobials, was not demonstrated in this study. However, Murano and Pierson (1993) heat shocked at 42°C for 5 min, which was substantially different than the 55°C for 10 min challenge conducted herein.

Sublethal levels of salt, or osmotic, stress prompted *Salmonella* Virchow to change morphotype and better survive, with the capability of reverting back to normal morphology following the removal of the stress (McMahon, McDowell et al. 2007). Others have shown that high salt, low- $a_w$  conditions may prompt *Salmonella* to enter a filamentous state to survive until favorable conditions return (Mattick, Jørgensen et al. 2000). In the present study, exposure to osmotic stress did not appear to enhance *Salmonella* survival when subsequently exposed to antimicrobials.

The USDA Food Safety and Inspection Service (FSIS) has established maximum concentrations of antimicrobials permitted for use in meat and poultry processing (USDA Food Safety and Inspection Service 2018). All of the MICs of non-stressed (control) strains obtained in this study (Table 2-11), and all MICs in general, were well below the concentrations permitted by USDA. The results shown here would be cause for concern had the observed MICs exceeded (or even approached) the maximum concentrations allowed by USDA. Because the observed MICs were so low and little change in MIC was observed following stress compared to control, decreased susceptibility to antimicrobials following stress has not been definitively shown here.

It is important to note, however, this study was performed *in vitro*. Once microbes are exposed to food/animal matrices the data presented herein may not apply directly. For example, food is a milieu of competitive bacteria, moisture limitations, and other growth-limiting factors, such as acid (Archer 1996). Indeed, foodborne bacteria encounter intrinsic (*i.e.*, the food matrix itself) and extrinsic (*e.g.*, water activity, pH, temperature) stressors throughout processing

(Buchanan and Doyle 1997, Chung, Bang et al. 2006). Nevertheless, this is a first look from a foundational standpoint that can inform future studies investigating the impact of stress on antimicrobial efficacy in a variety of food systems.

Some have recommended creating an alternating disinfection plan where different antimicrobials and mechanisms are rotated during the production week to prevent conjugated exchange of antimicrobial-resistant plasmids (Lemaitre, Echchannaoui et al. 1998). Although resistant species of bacteria were not obtained in this study, the recommendation is good nonetheless, even from a purely theoretical standpoint.

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## 2.7 TABLES & FIGURES

Plate 1

|   | 1                  | 2                  | 3                      | 4                      | 5                      | 6                      | 7                      | 8                      | 9                      | 10                     | 11                     | 12                     |
|---|--------------------|--------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| A | LaurArg 1<br>5 ppm | LaurArg 2<br>5 ppm | LaurArg 1<br>10<br>ppm | LaurArg 2<br>10<br>ppm | LaurArg 1<br>15<br>ppm | LaurArg 2<br>15<br>ppm | LaurArg 1<br>20<br>ppm | LaurArg 2<br>20<br>ppm | LaurArg 1<br>25<br>ppm | LaurArg 2<br>25<br>ppm | LaurArg 1<br>30<br>ppm | LaurArg 2<br>30<br>ppm |
| B | LaurArg 1<br>5 ppm | LaurArg 2<br>5 ppm | LaurArg 1<br>10<br>ppm | LaurArg 2<br>10<br>ppm | LaurArg 1<br>15<br>ppm | LaurArg 2<br>15<br>ppm | LaurArg 1<br>20<br>ppm | LaurArg 2<br>20<br>ppm | LaurArg 1<br>25<br>ppm | LaurArg 2<br>25<br>ppm | LaurArg 1<br>30<br>ppm | LaurArg 2<br>30<br>ppm |
| C | CA+HCl<br>1<br>1%  | CA+HCl<br>2<br>1%  | CA+HCl<br>1<br>1.25%   | CA+HCl<br>2<br>1.25%   | CA+HCl<br>1<br>1.5%    | CA+HCl<br>2<br>1.5%    | CA+HCl<br>1<br>1.75%   | CA+HCl<br>2<br>1.75%   | CA+HCl<br>1<br>2%      | CA+HCl<br>2<br>2%      | CA+HCl<br>1<br>2.25%   | CA+HCl<br>2<br>2.25%   |
| D | CA+HCl<br>1<br>1%  | CA+HCl<br>2<br>1%  | CA+HCl<br>1<br>1.25%   | CA+HCl<br>2<br>1.25%   | CA+HCl<br>1<br>1.5%    | CA+HCl<br>2<br>1.5%    | CA+HCl<br>1<br>1.75%   | CA+HCl<br>2<br>1.75%   | CA+HCl<br>1<br>2%      | CA+HCl<br>2<br>2%      | CA+HCl<br>1<br>2.25%   | CA+HCl<br>2<br>2.25%   |
| E | PAA+AA<br>1<br>1%  | PAA+AA<br>2<br>1%  | PAA+AA<br>1<br>1.25%   | PAA+AA<br>2<br>1.25%   | PAA+AA<br>1<br>1.5%    | PAA+AA<br>2<br>1.5%    | PAA+AA<br>1<br>1.75%   | PAA+AA<br>2<br>1.75%   | PAA+AA<br>1<br>2%      | PAA+AA<br>2<br>2%      | PAA+AA<br>1<br>2.25%   | PAA+AA<br>2<br>2.25%   |
| F | PAA+AA<br>1<br>1%  | PAA+AA<br>2<br>1%  | PAA+AA<br>1<br>1.25%   | PAA+AA<br>2<br>1.25%   | PAA+AA<br>1<br>1.5%    | PAA+AA<br>2<br>1.5%    | PAA+AA<br>1<br>1.75%   | PAA+AA<br>2<br>1.75%   | PAA+AA<br>1<br>2%      | PAA+AA<br>2<br>2%      | PAA+AA<br>1<br>2.25%   | PAA+AA<br>2<br>2.25%   |
| G |                    |                    |                        |                        |                        |                        |                        |                        |                        |                        |                        |                        |
| H | BLK                | BLK                |                        |                        |                        |                        |                        |                        |                        |                        |                        |                        |

Plate 1

|   | 1               | 2               | 3                | 4                | 5             | 6             | 7                | 8                | 9               | 10              | 11               | 12               |
|---|-----------------|-----------------|------------------|------------------|---------------|---------------|------------------|------------------|-----------------|-----------------|------------------|------------------|
| A | LA+CA 1<br>0.5% | LA+CA 2<br>0.5% | LA+CA 1<br>0.75% | LA+CA 2<br>0.75% | LA+CA 1<br>1% | LA+CA 2<br>1% | LA+CA 1<br>1.25% | LA+CA 2<br>1.25% | LA+CA 1<br>1.5% | LA+CA 2<br>1.5% | LA+CA 1<br>1.75% | LA+CA 2<br>1.75% |
| B | LA+CA 1<br>0.5% | LA+CA 2<br>0.5% | LA+CA 1<br>0.75% | LA+CA 2<br>0.75% | LA+CA 1<br>1% | LA+CA 2<br>1% | LA+CA 1<br>1.25% | LA+CA 2<br>1.25% | LA+CA 1<br>1.5% | LA+CA 2<br>1.5% | LA+CA 1<br>1.75% | LA+CA 2<br>1.75% |
| C | LA 1<br>0.3%    | LA 2<br>0.3%    | LA 1<br>0.4%     | LA 2<br>0.4%     | LA 1<br>0.5%  | LA 2<br>0.5%  | LA 1<br>0.6%     | LA 2<br>0.6%     | LA 1<br>0.7%    | LA 2<br>0.7%    | LA 1<br>0.8%     | LA 2<br>0.8%     |
| D | LA 1<br>0.3%    | LA 2<br>0.3%    | LA 1<br>0.4%     | LA 2<br>0.4%     | LA 1<br>0.5%  | LA 2<br>0.5%  | LA 1<br>0.6%     | LA 2<br>0.6%     | LA 1<br>0.7%    | LA 2<br>0.7%    | LA 1<br>0.8%     | LA 2<br>0.8%     |
| E |                 |                 |                  |                  |               |               |                  |                  |                 |                 |                  |                  |
| F |                 |                 |                  |                  |               |               |                  |                  |                 |                 |                  |                  |
| G | POS             | POS             |                  |                  |               |               |                  |                  |                 |                 |                  |                  |
| H |                 |                 |                  |                  |               |               |                  |                  |                 |                 |                  |                  |

**Figure 2.1: Sample plate layout with blanks and controls.**

Antimicrobials are LaurArg (lauric arginate), CA+HCl (citric acid and hydrochloric acid), PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), LA+CA (lactic acid and citric acid), and LA (lactic acid). Number following the antimicrobial is the duplicate identifier. Blue = bacteria added. Grey = Sanitizer blank control. BLK = Mueller-Hinton blank. POS = Positive control.

**Table 2-1: Concentration range of tested antimicrobials**

| <b>Antimicrobial</b> | <b>Range tested</b> |
|----------------------|---------------------|
| LaurArg              | 5 – 40 ppm          |
| CA+HCl               | 1.25 – 3.00%        |
| PAA+AA               | 90 – 220 ppm        |
| LA+CA                | 0.50 – 2.50%        |
| LA                   | 0.50 – 2.00%        |

**Table 2-2: *Salmonella* Montevideo MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | >30 ppm              | 2.25%         | 120 ppm       | 0.90%        | 0.80%     |
| <b>Acid</b>        | >30 ppm              | 2.25%         | 110 ppm       | 0.80%        | 0.80%     |
| <b>Alkaline</b>    | >30 ppm              | 2.25%         | 130 ppm       | 0.80%        | 0.80%     |
| <b>Freeze-Thaw</b> | >30 ppm              | 2.50%         | 110 ppm       | 0.90%        | 0.80%     |
| <b>Heat</b>        | 30 ppm               | 2.00%         | 90 ppm        | 0.70%        | 0.70%     |
| <b>Salt</b>        | >30 ppm              | 2.25%         | 130 ppm       | 0.80%        | 0.70%     |

**Table 2-3: *Salmonella* Newport MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 30 ppm               | 2.25%         | 120 ppm       | 0.90%        | 0.80%     |
| <b>Acid</b>        | 30 ppm               | 2.25%         | 130 ppm       | 0.90%        | 0.80%     |
| <b>Alkaline</b>    | >30 ppm              | 2.50%         | 110 ppm       | 0.90%        | 0.80%     |
| <b>Freeze-Thaw</b> | 30 ppm               | 2.25%         | 120 ppm       | 0.90%        | 0.80%     |
| <b>Heat</b>        | 30 ppm               | 2.00%         | 100 ppm       | 0.70%        | 0.70%     |
| <b>Salt</b>        | 30 ppm               | 2.25%         | 130 ppm       | 0.90%        | 0.80%     |

**Table 2-4: *Salmonella* Typhimurium MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 30 ppm               | 2.25%         | 130 ppm       | 0.90%        | 0.80%     |
| <b>Acid</b>        | 30 ppm               | 2.25%         | 110 ppm       | 0.80%        | 0.70%     |
| <b>Alkaline</b>    | 30 ppm               | 2.25%         | 110 ppm       | 0.90%        | 0.70%     |
| <b>Freeze-Thaw</b> | 30 ppm               | 2.25%         | 110 ppm       | 0.90%        | 0.70%     |
| <b>Heat</b>        | 30 ppm               | 2.00%         | 90 ppm        | 0.80%        | 0.70%     |
| <b>Salt</b>        | 30 ppm               | 2.25%         | 130 ppm       | 0.90%        | 0.80%     |

**Table 2-5: STEC O26 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 22.5 ppm             | 1.75%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Acid</b>        | 25 ppm               | 1.50%         | 100 ppm       | 0.75%        | 0.70%     |
| <b>Alkaline</b>    | 25 ppm               | 1.50%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Freeze-Thaw</b> | 25 ppm               | 1.75%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Heat</b>        | 25 ppm               | 1.50%         | 100 ppm       | 0.50%        | 0.70%     |
| <b>Salt</b>        | 25 ppm               | 1.75%         | 100 ppm       | 0.75%        | 0.70%     |

**Table 2-6: STEC O45 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 30 ppm               | 1.75%         | 120 ppm       | 0.80%        | 0.80%     |
| <b>Acid</b>        | 30 ppm               | 2.50%         | 130 ppm       | 0.75%        | 0.80%     |
| <b>Alkaline</b>    | 27.5 ppm             | 2.25%         | 140 ppm       | 0.70%        | 0.80%     |
| <b>Freeze-Thaw</b> | 27.5 ppm             | 2.00%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Heat</b>        | 27.5 ppm             | 1.75%         | 110 ppm       | 0.75%        | 0.60%     |
| <b>Salt</b>        | 27.5 ppm             | >2.50%        | 120 ppm       | 0.75%        | 0.70%     |

**Table 2-7: STEC O103 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 25 ppm               | >2.50%        | 110 ppm       | 0.75%        | 0.80%     |
| <b>Acid</b>        | 25 ppm               | >2.50%        | 120 ppm       | 0.80%        | 0.70%     |
| <b>Alkaline</b>    | 25 ppm               | 2.50%         | 130 ppm       | 0.75%        | 0.90%     |
| <b>Freeze-Thaw</b> | 30 ppm               | 2.50%         | 120 ppm       | 0.75%        | 0.80%     |
| <b>Heat</b>        | 25 ppm               | 1.75%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Salt</b>        | 25 ppm               | 2.50%         | 120 ppm       | 0.75%        | 0.80%     |

**Table 2-8: STEC O111 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 25 ppm               | 2.25%         | 100 ppm       | 0.75%        | 0.60%     |
| <b>Acid</b>        | 30 ppm               | 2.25%         | 120 ppm       | 0.75%        | 0.60%     |
| <b>Alkaline</b>    | 25 ppm               | 2.25%         | 110 ppm       | 0.75%        | 0.60%     |
| <b>Freeze-Thaw</b> | 27.5 ppm             | 2.50%         | 110 ppm       | 0.75%        | 0.70%     |
| <b>Heat</b>        | 25 ppm               | 2.00%         | 100 ppm       | 0.75%        | 0.50%     |
| <b>Salt</b>        | 22.5 ppm             | 2.50%         | 110 ppm       | 0.75%        | 0.60%     |

**Table 2-9: STEC O145 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 25 ppm               | >2.50%        | 140 ppm       | 0.75%        | 0.80%     |
| <b>Acid</b>        | 25 ppm               | >2.50%        | 130 ppm       | 1.00%        | 0.80%     |
| <b>Alkaline</b>    | 25 ppm               | >2.50%        | 130 ppm       | 0.75%        | 0.80%     |
| <b>Freeze-Thaw</b> | 25 ppm               | >2.50%        | 140 ppm       | 0.75%        | 0.80%     |
| <b>Heat</b>        | 25 ppm               | 1.75%         | 110 ppm       | 0.75%        | 0.80%     |
| <b>Salt</b>        | 22.5 ppm             | 2.50%         | 120 ppm       | 0.75%        | 0.70%     |

**Table 2-10: STEC O157 MICs as determined by LS Means**

|                    | <b>Antimicrobial</b> |               |               |              |           |
|--------------------|----------------------|---------------|---------------|--------------|-----------|
|                    | <b>LaurArg</b>       | <b>CA+HCl</b> | <b>PAA+AA</b> | <b>LA+CA</b> | <b>LA</b> |
| <b>Control</b>     | 40 ppm               | 2.25%         | 110 ppm       | 0.75%        | 1.00%     |
| <b>Acid</b>        | 40 ppm               | 2.25%         | 100 ppm       | 0.75%        | 1.00%     |
| <b>Alkaline</b>    | 40 ppm               | 2.25%         | 100 ppm       | 0.75%        | 1.00%     |
| <b>Freeze-Thaw</b> | 40 ppm               | 2.25%         | 110 ppm       | 0.75%        | 1.00%     |
| <b>Heat</b>        | 40 ppm               | 2.00%         | 90 ppm        | 0.75%        | 1.00%     |
| <b>Salt</b>        | 40 ppm               | 2.00%         | 130 ppm       | 0.75%        | 1.00%     |

**Table 2-11: Comparison of control MICs obtained and maximum amount of antimicrobial allowed by USDA**

|                                      | <b>Antimicrobial</b> |                               |               |                    |           |
|--------------------------------------|----------------------|-------------------------------|---------------|--------------------|-----------|
|                                      | <b>LaurArg</b>       | <b>CA+HCl</b>                 | <b>PAA+AA</b> | <b>LA+CA</b>       | <b>LA</b> |
| <b>Maximum allowed by USDA</b>       | 200 ppm <sup>†</sup> | determined by pH <sup>^</sup> | 400 ppm       | 2.50% <sup>†</sup> | 5.00%     |
| <b><i>Salmonella</i> Montevideo</b>  | >30 ppm              | 2.25%                         | 120 ppm       | 0.90%              | 0.80%     |
| <b><i>Salmonella</i> Newport</b>     | 30 ppm               | 2.25%                         | 120 ppm       | 0.90%              | 0.80%     |
| <b><i>Salmonella</i> Typhimurium</b> | 30 ppm               | 2.25%                         | 130 ppm       | 0.90%              | 0.80%     |
| <b>STEC O26</b>                      | 22.5 ppm             | 1.75%                         | 110 ppm       | 0.75%              | 0.70%     |
| <b>STEC O45</b>                      | 30 ppm               | 1.75%                         | 120 ppm       | 0.80%              | 0.80%     |
| <b>STEC O103</b>                     | 25 ppm               | >2.50%                        | 110 ppm       | 0.75%              | 0.80%     |
| <b>STEC O111</b>                     | 25 ppm               | 2.25%                         | 100 ppm       | 0.75%              | 0.60%     |
| <b>STEC O145</b>                     | 25 ppm               | >2.50%                        | 140 ppm       | 0.75%              | 0.80%     |
| <b>STEC O157</b>                     | 40 ppm               | 2.25%                         | 110 ppm       | 0.75%              | 1.00%     |

<sup>†</sup> applied at a level not to exceed said concentration by weight in the finished product

<sup>^</sup> a percent of solution was used *in lieu* of pH to replicate increasing concentrations for MICs

## **Chapter 3 - Attachment of Shiga Toxin-producing *Escherichia coli* (STEC) to Pre-Chill and Post-Chill Beef**

### **3.1. ABSTRACT**

Shiga toxin-producing *Escherichia coli* (STEC) has been implicated in beef-related foodborne illness outbreaks, and understanding bacterial attachment may inform future interventions at the abattoir. This study quantified STEC attachment under simulated meat processing conditions on adipose and lean beef tissue. Beef brisket samples were warmed to a surface temperature of 30°C, while the remaining samples were maintained at 4°C, prior to inoculation with STEC cocktail (O26, O45, O103, O111, O121, O145, and O157:H7; ca. 7 log CFU/mL) onto the meat surface. Cocktails were grown in either tryptic soy broth (TSB) or M9 minimal nutrient medium. Loosely attached and firmly attached cells were spread plated onto MacConkey agar at times 0, 3, 5, and 20 min and 1, 3, 8, 12, 24 and 48 h. At every sampling point, the meat sample was shaken for 90 s in a stomacher bag with 0.1% peptone water (PW; loosely attached cells), transferred into a second stomacher bag with fresh PW, and homogenized (firmly attached cells). When grown in TSB, time × sample type (buffer versus homogenized sample) was significant ( $p < 0.001$ ), as STEC cells became more firmly attached throughout storage. Sample type × tissue type (adipose versus lean) was statistically significant ( $p < 0.001$ ) indicating a difference in loose versus firmly attached populations on lean and adipose tissues. For STEC cells grown in M9, tissue type was a significant main effect ( $p = 0.0134$ ); however, the difference in attachment to lean versus adipose tissue was only 0.16 log<sub>10</sub> CFU/cm<sup>2</sup>. Time was also a significant main effect ( $p < 0.001$ ) due to variability in populations throughout the 48 h sampling period. Future research should investigate if an increase in firmly attached STEC cells

is correlated to reduced intervention efficacy on post-chill carcasses and subprimal cuts, as is commonly observed.

### 3.2. INTRODUCTION

The Centers for Disease Control and Prevention (CDC) estimates that *Escherichia coli* O157:H7 causes 96,000 illnesses annually, while an additional 168,000 annual illnesses are caused by the non-O157 *E. coli* serogroups O26, O45, O103, O111, O121, and O145 (known as the “Big Six”) (Scallan, Hoekstra et al. 2011). These organisms’ ability to produce Shiga toxins, which have serious health implications in humans, is of concern to human health. For this reason, the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) declared *E. coli* O157:H7 an adulterant in raw ground beef in 1994, and expanded adulterant status to non-intact meat and trimmings in 1999 (Taylor 1994, USDA Food Safety and Inspection Service 1999). In 2012, adulterant status was additionally declared for the “Big Six” serogroups in non-intact beef (USDA Food Safety and Inspection Service 2012). STEC are associated with beef cattle and are often present at the time of processing (Ferens and Hovde 2011). One way to mitigate the likelihood of STEC presence in beef products is to better understand the attachment of STEC on surfaces during harvest and fabrication (including the beef itself), knowledge of which may translate into new practices for STEC mitigation in beef processing.

Bacterial attachment processes and mechanisms are important concepts for food safety professionals, as attachment impacts bacterial presence in the food supply. The process of cellular attachment is complex: interactions at the molecular level are not fully understood and many factors can impact cellular adherence (Palmer, Flint et al. 2007). While studies examining *E. coli* attachment under beef processing conditions are limited (Skandamis, Stopforth et al.

2009, Dourou, Beauchamp et al. 2011), several factors—both extrinsic and intrinsic—are of importance.

Extrinsically, a major factor is the temperature of the system. It has been demonstrated that temperature impacts the growth and survival of STEC in beef (Tamplin, Paoli et al. 2005). Growth media is another extrinsic factor that can impact *E. coli* attachment to surfaces. As an example, M9 minimal salt medium—a starvation/stress medium—has been used to create an environment that may simulate conditions as they occur in processing and cleaning practices (Parks and Brashears 2015). The meat surface itself—lean or adipose tissue—is also an extrinsic factor that may impact bacterial attachment. However, the overall research consensus is that bacteria attach equally to lean and adipose tissue, with variation occurring only within 0.5 logs (Frank 2001).

Intrinsically, cellular appendages and surface proteins can impact bacterial attachment (Goulter, Gentle et al. 2009, McWilliams and Torres 2014). This includes the capsule, fimbriae, outer membrane polymers, the surface layer, and other attachment organelles (Frank 2001). Surface charge and hydrophobicity of the bacterial cell (Dickson and Koohmaraie 1989, Goulter, Gentle et al. 2009) are additional intrinsic factors that contribute to bacterial attachment. For lean tissue, initial bacterial cell attachment involves interactions with negative charges on the bacterial cell surface (Dickson and Koohmaraie 1989). For adipose tissue attachment, bacterial cellular hydrophobicity likely impacts attachment strength and population (Pringle and Fletcher 1983, Dickson 1991).

This study's goal was to elucidate the attachment rate of STEC onto beef tissues over the course of 48 h under a variety of conditions that simulate beef harvest and postharvest processes.

An additional goal was to compare the attachment of STEC grown in a minimal nutrient medium to metabolically active STEC grown in a nutrient-dense growth medium.

### 3.3. MATERIALS AND METHODS

**Experimental design.** Lean and adipose tissue samples were obtained from vacuum-packaged, chilled beef briskets. Prior to inoculation, samples of both tissue types were stored at 30°C (warm samples) and 4°C (cold samples) to simulate STEC contamination on the pre-chill and post-chill carcass, respectively. Samples were surface-inoculated and populations of loosely attached and firmly attached STEC cells were determined over the course of 48 h. All post-inoculation cold samples were immediately returned to storage at 4°C, while all post-inoculation warm samples were stored at 4°C after 30 min, which represents the approximate length of time the warm carcass is undergoing harvest prior to entering the cooler. Attachment was determined over the course of 48 h to understand STEC attachment throughout the carcass chilling process.

**Beef sample preparation.** Beef briskets were purchased from a local grocer. Prior to analysis, a thin layer was removed from the brisket surface with a sterile knife in order to remove any residual carcass or fabrication interventions that may interfere with bacterial attachment and/or survival. From this primal cut, 50 cm<sup>2</sup> samples of adipose (n=20) and lean (n=20) tissue were collected for each replication. Meat samples were separated by lean and adipose tissue and stored in poultry rinse bags at 4°C for 18-24 h before inoculation.

**Culture preparation.** Frozen stock cultures of *Escherichia coli* O157:H7 and serogroups O26, O45, O103, O111, O121, and O145, stored in tryptic soy broth (TSB; BD Difco, Franklin Lakes, NJ) at -80°C, were used to prepare a cocktail for inoculation. Briefly, each frozen isolate was streaked for isolation onto tryptic soy agar (TSA; Remel, Lenexa, KS) and incubated at 37°C for 18-24 h. From each plate, a single isolated colony was transferred to 9

mL of TSB or M9 minimal salts medium (M9; BD Difco, Franklin Lakes, NJ) and grown at 37°C for 24 h. M9 medium was prepared by supplementing 200 mL M9 minimal salts solution (5x) with 20 mL glucose (20%; Fisher Chemical, Fair Lawn, NJ), 2 mL MgSO<sub>4</sub> (1.0 M; Fisher Chemical, Fair Lawn, NJ), 0.1 mL CaCl<sub>2</sub> (1.0 M; Fisher Chemical, Fair Lawn, NJ) and 750 mL deionized water. Following the 24 h incubation, TSB and M9 culture tubes were centrifuged at  $5,000 \times g$  for 15 min at 4°C. The supernatant was discarded and the pellet was re-suspended in 9 mL of 0.1% peptone water (PW; BD Difco, Franklin Lakes, NJ). Resuspended inoculum tubes were combined in equal proportions to prepare an inoculum cocktail, which was diluted in PW to achieve the desired starting titer of ca. 5.0 log<sub>10</sub> CFU/mL.

**Beef tissue attachment assay.** On day 0 of the study, all 50 cm<sup>2</sup> adipose and lean tissue samples were removed from 4°C storage and randomly assigned to either the warm or refrigerated treatment group. Samples assigned to the warm treatment were heated to a surface temperature of 30°C (simulating a warm carcass surface temperature during harvest) in an incubator while cold samples remained at 4°C until inoculation. Immediately prior to inoculation, all samples were placed into a sterile 11.5- × 9.5- × 2-inch (29.21- × 24.13- × 5.08-cm) half hotel pan with a metal grate placed on the bottom, such that samples were not in direct contact with the base of the pan. Samples were surface-inoculated by pipetting 150 µL of the inoculum cocktail onto the meat surface, which was evenly dispersed with an “L-shaped” spreader (Fisher Science, Hampton, NH). Following inoculation, warm samples were stored for 30 min at 30°C, before being transferred to refrigeration temperature (4°C), while cold samples were transferred immediately in covered pans to 4°C refrigeration.

Sampling of inoculated meat samples was conducted at time points 0 min, 3 min, 5 min, 20 min, 1 h (60 min), 3 h (180 min), 8 h (480 min), 12 h (720 min), 24 h (1440 min), and 48 h

(2880 min). Time began when the STEC cocktail had been pipetted and spread onto the tissue surface using the cell spreader. Following inoculation, methods to release loosely and firmly attached cells from the tissue samples were based upon those previously described by Rivas et al. (Rivas, Fegan et al. 2007), with modifications. Briefly, each 50 cm<sup>2</sup> sample was transferred into a stomacher bag containing 250 mL of PW and placed into a shaking incubator set at 4°C and 200 rpm for 90 s (Excella E24 Incubator Shaker, New Brunswick Scientific, Edison, NJ). This process released loosely attached cells into the PW; therefore, bacterial populations obtained from this PW sample were counted as loosely attached and will be referred to hereafter as “loose.” Following shaking, the 50 cm<sup>2</sup> sample was aseptically transferred into a new stomacher bag containing 250 mL of fresh PW and homogenized (Stomacher® 400 Circulator, Seward, Bohemia, NY) for 60 s at 230 rpm. This process released firmly attached cells into the PW and bacterial populations obtained from this PW sample were counted as firmly attached and will hereafter be referred to as “firm”. All samples were serially diluted in PW, plated onto MacConkey agar (Remel; Lenexa, KS), and incubated at 37°C for 18-24 h.

**Statistical analysis.** All experimental procedures were replicated three times. Data collected from all three replications were analyzed using the MIXED procedure with LSMEANS statement of Statistical Analysis Software (SAS 9.4; Cary, NC). For each media type, the main effects (sample type, tissue type, temperature, time) and interactions were evaluated for statistical significance at the  $p \leq 0.05$  threshold. Data were analyzed for each media type individually; thus, media type was not included in the statistical model. Because all samples were placed into refrigerated storage at 30 min, data for the 0 min, 3 min, 5 min, and 20 min time points (all time points prior to refrigeration) were also analyzed separately to more clearly probe the relationship of initial STEC attachment and product temperature (warm versus cold).

### 3.4. RESULTS

**TSB-Grown STEC.** When STEC cultures were grown in TSB, time was the only significant main effect ( $p < 0.0001$ ). Regarding interactions, sample type (loose versus firm)  $\times$  time and sample type  $\times$  tissue type (lean versus adipose) were significant ( $p < 0.0001$  for both). Although time by itself was significant, this variable was included in the sample type  $\times$  time interaction; therefore, data will be shown in regards to sample type and time and will not be shown according to time alone. TSB-grown STEC became more firmly attached over time (Figure 3.1; Table 3-1). Prior to 60 min, loosely attached STEC were greater in population than firmly attached cells, with the disparity decreasing over time. At the 60 min time point, the loosely and firmly attached cells were most similar in population: a mere  $0.10 \log_{10} \text{ CFU/cm}^2$  difference, which was not statistically significant (Table 3-1;  $p = 0.3778$ ). Although firmly attached STEC populations were increasing while loosely attached populations were decreasing after 60 min, a statistical difference in population was not detected until 720 min (12 h), when the firmly attached STEC population was  $0.29 \log_{10} \text{ CFU/cm}^2$  larger ( $p = 0.0107$ ) than loosely attached STEC. At the end of the 2,880 min (48 h) storage period, firmly attached STEC were  $0.46 \log_{10} \text{ CFU/cm}^2$  greater than loosely attached STEC ( $p < 0.0001$ ). The sample type  $\times$  tissue type interaction that was detected for TSB-grown STEC suggests that STEC attaches differently to lean versus adipose tissue (Figure 3.2), with a larger population of firmly attached STEC recovered from the adipose tissue.

**M9-Grown STEC.** Tissue type ( $p = 0.0134$ ) and time ( $p < 0.0001$ ) were the significant main effects. As a result, all M9 data will be presented in regards to tissue type and time alone. When grown in M9, a nutrient-limiting media, STEC populations varied throughout the 2,880 min (48 h) storage period, with the largest discrepancy ( $0.89 \log \text{ CFU/cm}^2$ ) occurring between

populations at 480 min (8 h) and 2,880 min (48 h;  $p < 0.0001$ ). At time 0, the STEC population was  $3.60 \log_{10} \text{ CFU/cm}^2$ , which was similar to populations at all other time points, with the exception of 480 min and 2,880 min (Figure 3.3). More M9-grown STEC attached to adipose tissue than lean tissue ( $p = 0.0134$ ; Figure 3.4).

**Influence of Temperature on STEC Attachment.** Temperature was not a significant variable for either TSB- or M9-grown STEC ( $p > 0.05$ ). The TSB and M9 data collected for all time points prior to refrigeration at 30 min were also statistically analyzed separately from the remaining time points (up to 30 min, or, through the 20 min sampling point as no sampling occurred at 30 min specifically). Temperature was not a significant variable ( $p > 0.05$ ) for TSB-grown STEC attachment within the first 20 min post-inoculation. The main effect of sample type ( $p < 0.0001$ ) and the sample type  $\times$  tissue type ( $p = 0.0034$ ) interaction were significant within the first 20 min; however, because sample type is included in the interaction, sample type data will only be shown as it pertains to tissue type. More TSB-grown STEC firmly attached to adipose tissue than to the lean tissue ( $p = 0.0020$ ); however, the difference in populations was a modest  $0.24 \log \text{ CFU/cm}^2$  (Figure 3.5). The largest difference in population ( $0.64 \log \text{ CFU/cm}^2$ ) was observed between firmly attached and loosely attached STEC on lean tissues ( $p < 0.0001$ ).

STEC cells grown in M9 did not demonstrate any significant main effects or interactions ( $P > 0.05$ ) within the first 20 min post-inoculation. Thus, temperature was not a significant variable for M9-grown STEC attachment within the first 20 min post-inoculation.

### 3.5. DISCUSSION

**TSB-Grown STEC.** Longer durations of storage often result in increased attachment rates, particularly when duration shifts from hours to days (Dourou, Beauchamp et al. 2011). TSB-grown STEC become more firmly attached to beef as time progresses (Table 3-1, Figure

3.1), which is consistent with previously published findings. Figure 3.2 illustrates that STEC more firmly attach to adipose tissue than lean tissue and, subsequently, a larger population of loosely attached STEC was recovered from lean beef tissue. While it is expected that adipose tissue would be less hydrophobic, a study examining the ability of *S. Typhimurium* to attach to chicken surfaces concluded that damaged fatty cells may result in a “fatty coating” that creates an enhanced hydrophobic surface, resulting in an increase of bacterial adhesion to beef tissue (Dickson and Koohmaraie 1989). In the present study, the greatest difference in population was 0.26 log CFU/cm<sup>2</sup>, which was detected between STEC firmly attached versus loosely attached to lean tissue ( $p < 0.0001$ ). While this difference is statistically significant, it is not of great magnitude from a biological sense. In general, the TSB-grown STEC data are in agreement with the literature that STEC attachment is similar on lean and adipose beef tissue (Dickson and Koohmaraie 1989, Dickson 1991, Dickson and Frank 1993, Cabedo, Sofos et al. 1997).

Previously published research indicates that other pathogens behave similarly to STEC when attaching to lean and adipose beef tissues. *Salmonella choleraesuis* subsp. *choleraesuis* better adhered to lean versus adipose tissue, although the difference in attachment was less than 0.4 logs (Bouttier, Linxe et al. 1997). A survey of attachment involving *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella arizonae*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* to lean and adipose tissue showed no significant difference in populations for any of the organisms (Chung, Dickson et al. 1989). Contaminating beef tissue by cattle manure inoculated with *Salmonella* Typhimurium and *L. monocytogenes* resulted in similar attachment patterns between lean and adipose tissue and population differences were insignificant or less than 0.5 logs (Dickson and MacNeil 1991).

**M9-Grown STEC.** The largest population of M9-grown STEC was recovered from the 2,880 min (48 h) time point, suggesting that STEC populations eventually grew towards the end of storage. The M9-grown STEC underwent consecutive stressors: nutrient limitations in M9 media followed by cold (4°C) storage temperature, and this “double stress”, followed by the selectivity of MacConkey agar used for enumeration, may have impacted populations. Therefore, it is possible that the increased population at 2,880 min (48 h) occurred as a result of STEC acclimating to, and overcoming, the combination of stressful environments. It is expected that the refrigeration temperature would hinder the growth of STEC, as previous research has demonstrated that refrigeration extends the length or presence of lag phase growth (Tamplin, Paoli et al. 2005). Thus, rather than definitively concluding that STEC grew during storage at 4°C, it is important to also recognize that this discrepancy in population might have been the result of difficulty enumerating injured/stressed cells prior to the 2,880 min sampling point.

Figure 3.4 illustrates that larger populations of M9-grown STEC attached to adipose tissue than lean tissue; however, the difference in attachment was 0.16 log CFU/cm<sup>2</sup>, which is negligible from a biological sense. Therefore, although they are statistically different, these data are not particularly informative in regards to understanding STEC attachment on beef tissues.

Growing STEC cells in M9 medium was intended to replicate the stress that STEC undergo on the hide, and/or during beef processing and fabrication, which may provide a better representation of how STEC might attach in real-world scenarios. It has been documented that manipulation of growth conditions can affect STEC’s ability to attach to surfaces such as stainless steel (Rivas, Fegan et al. 2007), and the present study suggests this is also true for STEC attachment to beef tissue surfaces.

When bacteria are grown in “starvation stress” media, the population of bacteria (*Listeria monocytogenes*, *Salmonella typhimurium*, and *E. coli* O157:H7) attached to lean and adipose beef tissue decreased in some studies (Dickson and Frank 1993). Cells that are able to survive starvation have exhibited a decreased ability to attach to beef, although not significantly (Dickson and Frank 1993), and cellular stress induced by such a growth medium (such as a minimal salt medium) may influence cellular attachment (Patel, Sharma et al. 2011). Although attachment of TSB- and M9-grown STEC were not statistically compared, it was generally observed that M9-grown STEC attachment was more inconsistent than TSB-grown STEC, which supports previously published studies that cellular stress influences bacterial cell attachment.

**Influence of Temperature on STEC Attachment.** Data collected from time points 0 min through 2,880 min (48 h) suggest that temperature did not impact TSB- or M9-grown STEC attachment to adipose or lean tissue beef surfaces. The impact of temperature may have been confounded by the experimental design, as all warm samples were placed into refrigerated storage at 30 min post-inoculation, while all cold samples were maintained at 4°C throughout the study. Separate statistical analyses were conducted for data collected from time points 0 min through 20 min (all samples were refrigerated 30 min post-inoculation) in order to address this potentially confounding factor. Although the impact of refrigerated storage 30 min post-inoculation was removed from analyses, temperature (warm versus cold samples) was not a significant variable ( $p>0.05$ ), nor was it included in any significant interactions. Thus, it can be concluded that initial temperature of either tissue type did not impact STEC attachment within the first 20 min in this study.

The largest populations of TSB-grown STEC were loosely attached to lean and adipose tissues within the first 20 min post-inoculation (Figure 3-5). The fact that the largest population

difference occurred between loosely and firmly attached STEC on lean tissue is in agreement with the trend observed when data from all TSB-grown STEC time points (0 min through 2,880 min) were analyzed in the same statistical model.

Tissue type and time were significant main effects when M9 data from all time points (0 min through 2,880 min) were analyzed in the same statistical model. Similarly, tissue type ( $p=0.0906$ ) and time ( $p=0.1019$ ) represented the most significant variables within the first 20 min; however, they were not considered statistically significant in regards to the previously determined  $p \leq 0.05$  threshold. Because temperature was not a significant variable within 20 min post-inoculation, or throughout the entire 2,880 min (48 h) observation period, it can be concluded that temperature of beef tissues does not impact the attachment of M9-grown STEC.

Some studies have reported mixed results regarding the impact of temperature on bacterial attachment. For example, researchers have shown that growth temperatures may impact attachment on adipose tissue but not lean tissue (Dickson 1991). When cells were grown in nutrient-limiting conditions, attachment decreased as temperature increased, although not to a statistically significant level (Dickson and Frank 1993). In another study, both temperature and tissue type did not demonstrate statistically significant differences in *E. coli* populations (Dickson and Frank 1993).

Researchers have demonstrated that *E. coli* O157:H7 can attach to stainless steel and high-density polyethylene surfaces at temperatures associated with “non-production hours” (i.e., 15°C) and cold storage (i.e., 4°C), and the attachment ability increases at both of these temperatures over time (Dourou, Beauchamp et al. 2011). While the present study was not investigating these artificial surfaces, this study supports previously published data that STEC attachment increases over time at refrigerated and non-refrigerated temperatures.

**Summary.** Firmly attached STEC cells increase throughout time, especially when STEC cells originate from TSB. STEC cells originating from M9 displayed variable attachment, which suggests that the metabolic state of STEC influences the ability of cells to adhere to beef surfaces. In general, these data are in agreement with previously published research describing bacterial attachment to beef surfaces. While a body of evidence currently exists on how factors like temperature, stress, and tissue type impact bacterial attachment, the present study is unique in that it incorporates all of these factors to understand how a cocktail of *E. coli* O157:H7 and the “Big Six” adulterant serogroups attach during simulated beef harvest and postharvest processes.

Further understanding of the effect of the media of origin, and thus the environment immediately preceding contamination, is needed before data can be effectively used for future food safety practices at the abattoir. This is an important variable to consider, as bacterial cells, including STEC, that are entering the abattoir on the hide of an animal are not experiencing the same environmental conditions that optimal growth parameters typically used in the laboratory setting would provide. The attachment data described herein can inform future investigations designed to evaluate the possibility of reduced intervention efficacy of STEC on post-chill and subprimal cuts of beef.

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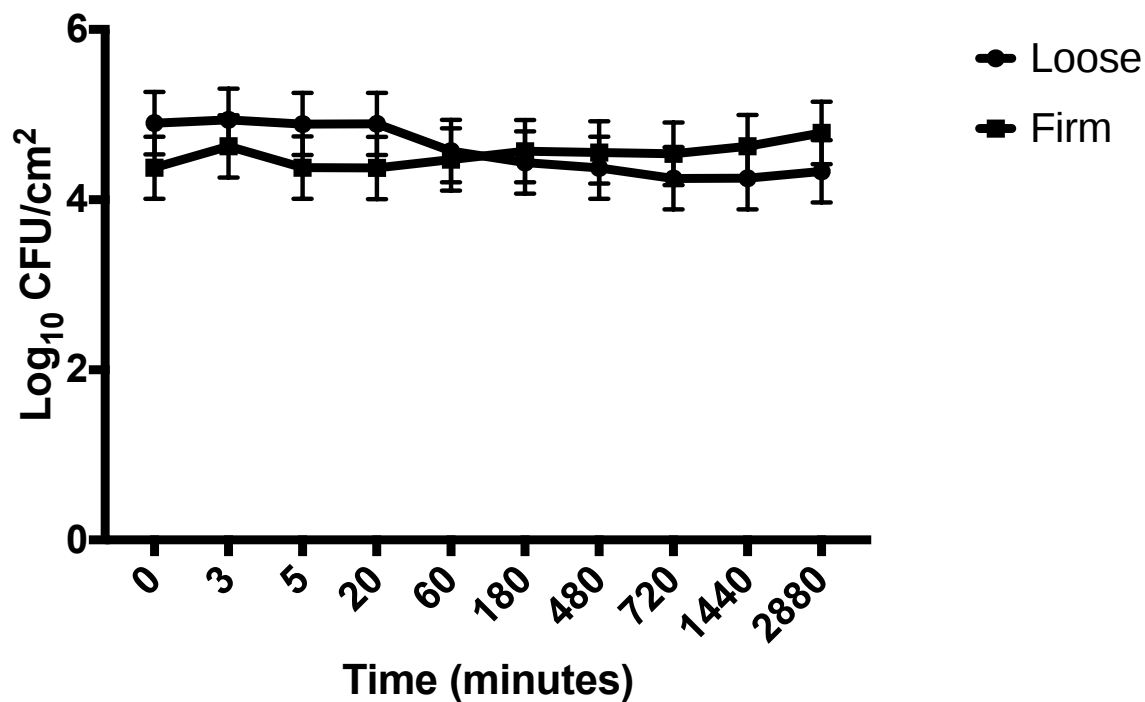
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### 3.7. TABLES & FIGURES

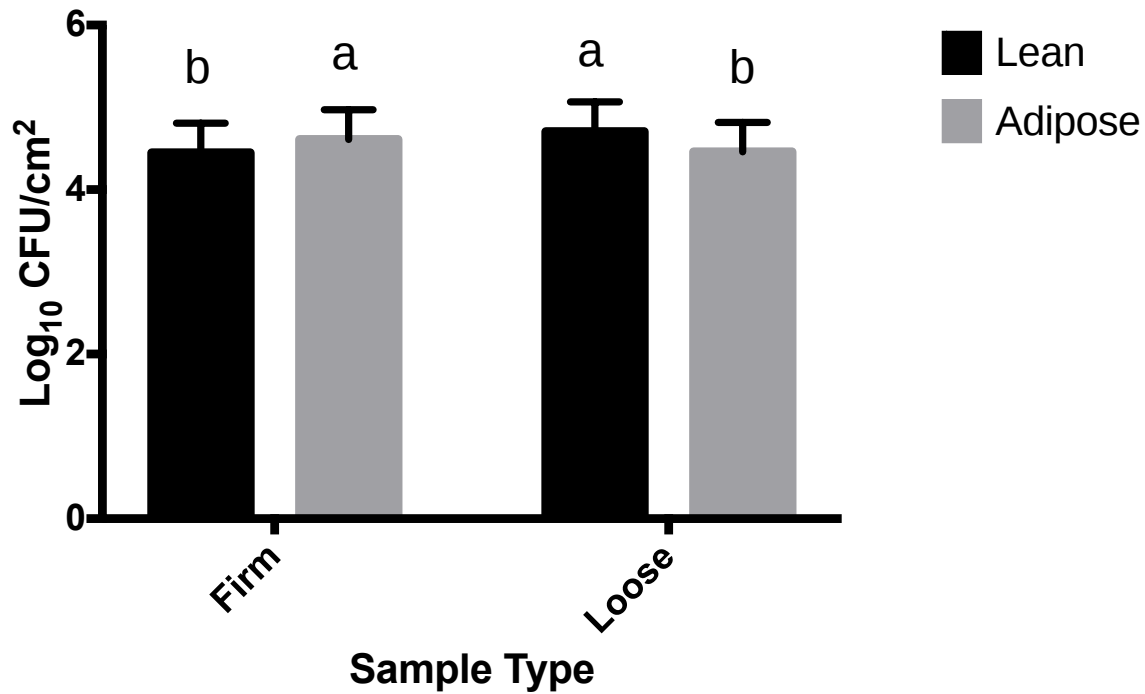
**Table 3-1: Populations of STEC cells grown in TSB represent loose and firm attachment to beef tissues during 48 h (2,880 min) of storage at 4°C. Time × sample type was significant (P<0.0001). Because time × sample type × tissue type interaction was not significant (P>0.05), data for adipose and lean tissue attachment are combined for each data type.**

| Time (min) | Beef Tissue Type                 |                                 |
|------------|----------------------------------|---------------------------------|
|            | Loose (log CFU/cm <sup>2</sup> ) | Firm (log CFU/cm <sup>2</sup> ) |
| 0          | 4.90 <sup>a</sup>                | 4.38 <sup>d,e,f</sup>           |
| 3          | 4.94 <sup>a</sup>                | 4.63 <sup>b,c</sup>             |
| 5          | 4.89 <sup>a</sup>                | 4.38 <sup>d,e,f</sup>           |
| 20         | 4.89 <sup>a</sup>                | 4.37 <sup>d,e,f</sup>           |
| 60         | 4.57 <sup>b,c,d</sup>            | 4.47 <sup>c,d,e</sup>           |
| 180        | 4.43 <sup>c,d,e,f</sup>          | 4.57 <sup>b,c,d</sup>           |
| 480        | 4.37 <sup>d,e,f</sup>            | 4.56 <sup>c,d</sup>             |
| 720        | 4.25 <sup>f</sup>                | 4.54 <sup>c,d,e</sup>           |
| 1440       | 4.25 <sup>f</sup>                | 4.63 <sup>b,c</sup>             |
| 2880       | 4.33 <sup>e,f</sup>              | 4.79 <sup>a,b</sup>             |

<sup>a,b,c,d,e,f</sup> Observations with different superscripts vary statistically. Observations can be compared across columns and rows.

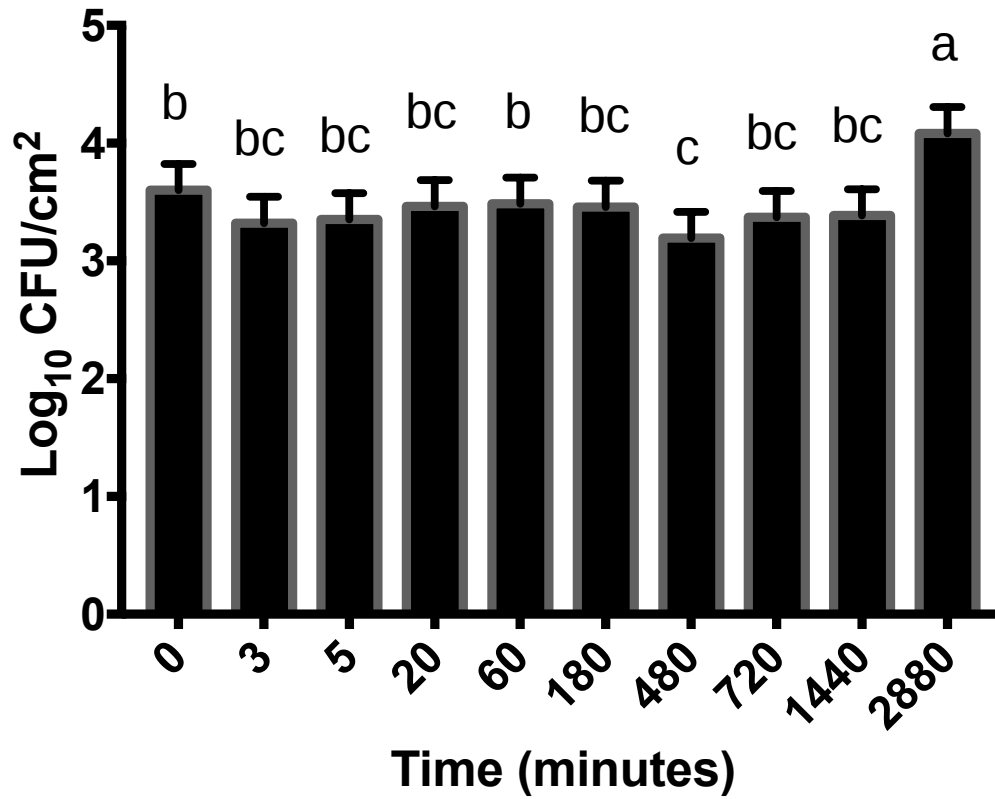


**Figure 3.1: Populations of TSB-grown STEC cells loosely and firmly attached to beef tissue during 48 h (2,880 min) of storage at 4°C. Time × sample type was significant ( $P < 0.0001$ ). Because the time × sample type × tissue type interaction was not significant, ( $P > 0.05$ ), data for adipose and lean tissue attachment are combined for each data point.**



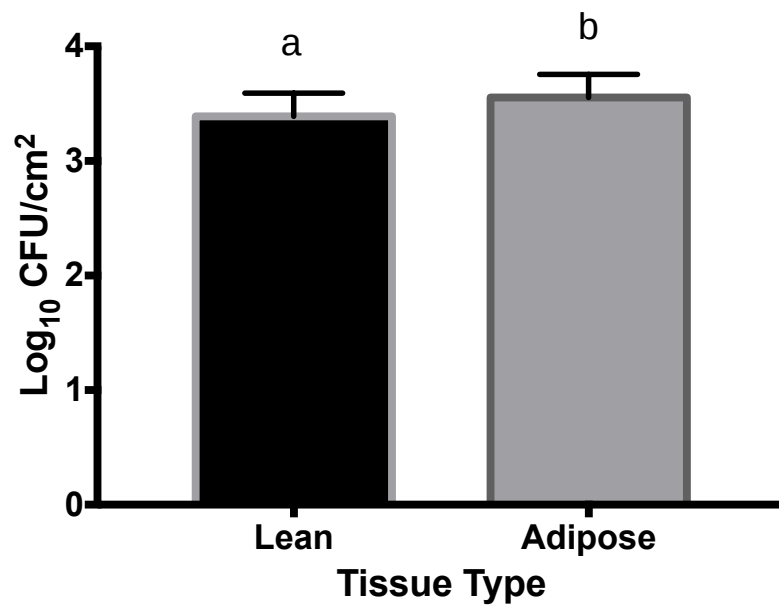
**Figure 3.2: Populations of firmly or loosely attached STEC cells by lean or adipose beef tissue. Sample type × tissue type was significant ( $P < 0.0001$ ). Because the time × sample type × tissue type interaction was not significant ( $P > 0.05$ ), data for each time point are combined.**

<sup>a,b</sup> Observations with different superscripts vary statistically.



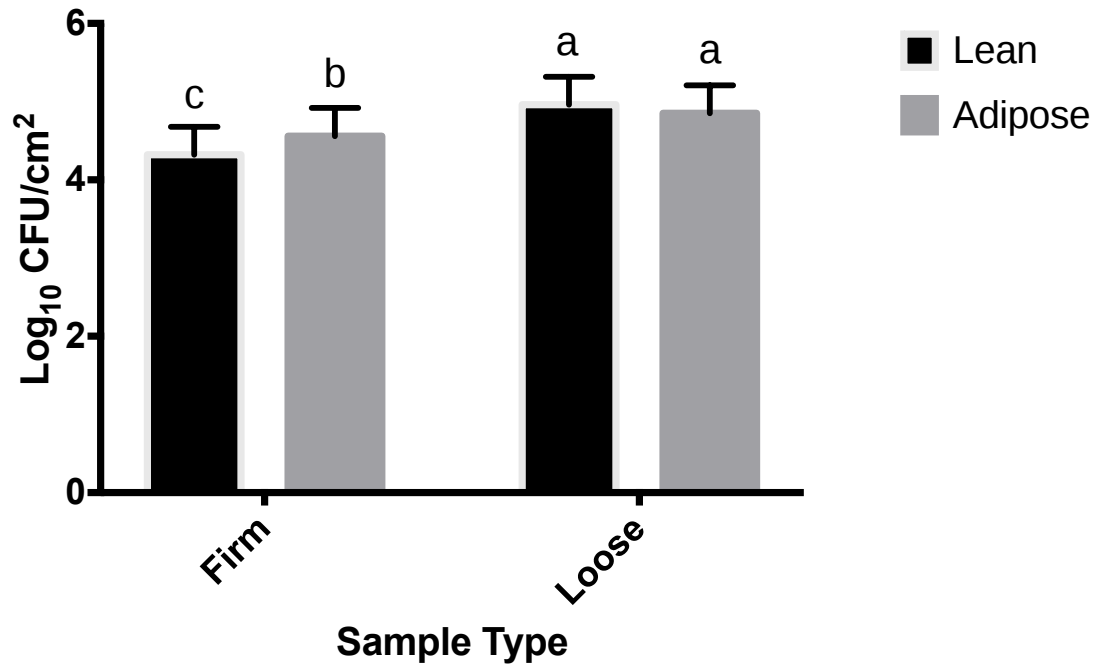
**Figure 3.3: Populations of STEC cells grown in M9 at each sampling point. The main effect of time was significant ( $P < 0.0001$ ). Because all variable interactions were not significant ( $P > 0.05$ ), all data for each time point are combined into a single observation.**

<sup>a,b,c</sup> Observations with different superscripts vary statistically.



**Figure 3.4: Populations of STEC cells grown in M9 by tissue type. The main effect of tissue type was significant ( $P < 0.0134$ ). Because all variable interactions were not significant ( $P > 0.05$ ), all data for each tissue type are combined into a single observation.**

<sup>a,b</sup> Observations with different superscripts vary statistically.



**Figure 3.5: Populations of TSB-grown STEC cells loosely and firmly attached to lean and adipose beef tissue within 20 min post-inoculation. Sample type × tissue type was significant ( $P < 0.0001$ ). Because the time × sample type × tissue type interaction was not significant ( $P > 0.05$ ), data for each time point are combined.**

<sup>a,b,c</sup> Observations with different superscripts vary statistically.

## Chapter 4 - Control of *Salmonella* on Fresh Spinach by Application of a Sodium Bisulfate/Peroxyacetic Acid Solution

### 4.1. ABSTRACT

Fresh produce has become a commonly-implicated product in foodborne illness outbreaks, including outbreaks of *Salmonella*. The use of antimicrobial washes is a common intervention to control pathogens in fresh-cut produce. While chlorine is a frequently used antimicrobial, it is only moderately efficacious on the product surface; thus, exploring other chemical interventions is warranted. This study quantifies the destruction of *Salmonella* spp. on fresh-cut spinach following a sodium bisulfate/peroxyacetic acid (SBS-PAA) wash and compares reductions to other interventions. Fresh-cut spinach was inoculated with a cocktail of *Salmonella* spp. at a target concentration of 5 log CFU/g, allowed to dry to facilitate attachment, and then submerged in one of three washes: SBS-PAA (80 ppm peroxyacetic acid plus 0.5% w/v sodium bisulfate), chlorine (150 ppm at pH 7.0), or water for 2 min, plus a non-washed control. Spinach was immediately sampled (d 0) or packaged in plastic retail display bags and stored at 7°C for 1, 3, 5, and 10 d until sampling. At each sampling point, *Salmonella* populations were enumerated using xylose lysine tergitol-4 (XLT-4) agar, or XLT-4 agar plus a tryptic soy agar (TSA) overlay to enumerate injured populations, and then incubated at 37°C for 18 h. When plated on XLT-4, SBS-PAA and chlorine washes achieved significant reductions compared to water wash and control ( $p \leq 0.05$ ). SBS-PAA and chlorine were similar ( $p > 0.05$ ) in efficacy. When plated on XLT-4 plus TSA overlay, all treatments differed from the control ( $p < 0.05$ ). SBS-PAA was the most effective, with a reduction of 1.77 log CFU/g ( $p < 0.0001$ ) and 0.46 log CFU/g ( $p = 0.0270$ ) in comparison to the control and chlorine washes, respectively. An SBS-PAA wash significantly

reduced *Salmonella* populations on fresh-cut spinach and may serve as an alternative to traditional chlorine washes.

## 4.2. INTRODUCTION

The implication of contaminated produce in foodborne illness outbreaks has been a major shift in foodborne illness source attribution (Brandl 2006). Foodborne illness caused by produce is a major public health concern, with norovirus and *Salmonella* spp. causing the most illness in the United States from 2004-2012 (Callejon, Rodriguez-Naranjo et al. 2015). Multistate produce outbreaks in the United States are often attributed to *Salmonella* (Callejon, Rodriguez-Naranjo et al. 2015), and *Salmonella* infections have been traced to tomatoes, melons, peppers, and sprouted seeds (Berger, Sodha et al. 2010). In an epidemiological study conducted by the Centers for Disease Control and Protection (CDC), Herman and colleagues (Herman, Hall et al. 2015) reported that contaminated leafy greens were implicated in 73.6% of foodborne illness outbreaks that occurred between the years of 1973-2012. Although not associated with *Salmonella*, an infamous outbreak traced to spinach occurred across 24 states in 2006 in which 191 confirmed *E. coli* O157:H7 cases resulted in high levels of hospitalizations (55%), hemolytic uremic syndrome (HUS; 17%) and deaths (2%) (Sharapov, Wendel et al. 2016).

The use of produce washes as an intervention to control microbial contamination on produce has been vastly explored in the literature (Beuchat, Ward et al. 2001, Harris, Beuchat et al. 2001, Luo, Nou et al. 2011, Banach, Sampers et al. 2015, Litt, Ravishankar et al. 2016, Dunkin, Weng et al. 2017). Chlorine is commonly used as a sanitizer for produce and produce wash water; however, efficacy at reducing pathogen populations on produce is not consistent (Neal, Marquez-Gonzalez et al. 2012). Additional work has explored the ramifications of repeated use of wash water and the resultant impacts on water quality, microbial survival, and

produce quality (Luo 2007). Specifically, organic matter can impact the disinfectant, lowering residual concentration and reducing efficacy of the sanitizer to eliminate microbes (Banach, Sampers et al. 2015). Chemicals such as peroxyacetic acid (PAA) have been suggested as alternatives to chlorine-based sanitizers (Banach, Sampers et al. 2015).

Peroxyacetic acid is a chemical that is comprised of acetic acid and hydrogen peroxide (Kitis 2004, Vandekinderen, Devlieghere et al. 2009). The production of reactive oxygen species (ROS) is responsible for the primary antimicrobial effect that PAA exerts on bacterial cells (Small, Chang et al. 2007, Vandekinderen, Devlieghere et al. 2009). These ROS damage lipids and DNA of the bacterial cell (Small, Chang et al. 2007, Vandekinderen, Devlieghere et al. 2009). However, PAA also denatures enzymes and proteins, increases permeability of the cell wall (Hilgren, Swanson et al. 2007, Small, Chang et al. 2007, Vandekinderen, Devlieghere et al. 2009), disturbs cell membranes, and blocks transport and enzymatic systems (Koivunen and Heinonen-Tanski 2005, Vandekinderen, Devlieghere et al. 2009). Peroxyacetic acid has been used as a fresh-cut produce wash water disinfectant to reduce yeasts and molds, which results in extended shelf-life and improved product quality (Neal, Marquez-Gonzalez et al. 2012). However, limited research exists regarding the effectiveness of PAA at reducing *Salmonella* populations on fresh-cut spinach.

When dissolved in water, sodium bisulfate dissociates into ions of sodium, hydrogen, and sulfate, which lowers the pH and creates an osmotic effect (Knueven 2013) that stresses bacterial cells. Slight declines in pH require enteric microorganisms to expend energy in order to regulate their cytoplasmic pH to maintain conditions that are near-neutral pH; however, this process stresses bacterial cells and will often lead to cell death (Hill, O'Driscoll et al. 1995, Laury, Alvarado et al. 2009). Previous research demonstrates that sodium bisulfate has reduced

*Salmonella* populations on chicken carcasses (Lj, Slavik et al. 1997); however, sodium bisulfate research to control pathogens on fresh-cut spinach is lacking.

The purpose of this study was to explore the efficacy of a postharvest wash solution consisting of 0.50% (w/v) sodium bisulfate and 80 ppm peroxyacetic acid at reducing *Salmonella* populations on fresh-cut spinach.

### 4.3. MATERIALS AND METHODS

**Culture preparation.** Frozen stock cultures of *Salmonella enterica* subsp. Anatum, Montevideo, Newport, and Typhimurium, stored in tryptic soy broth (TSB; BD Difco, Franklin Lakes, NJ) with 15% glycerol at -80°C, were used to prepare the inoculation cocktail. All *Salmonella* cultures were isolated from cattle and were originally obtained from researchers at Kansas State University. *Salmonella* was revived from frozen in 9 mL TSB at 37°C for 24 h, with two tubes prepared for each serotype (18 mL total). Following 24 h incubation, 18 mL of each serotype was centrifuged at  $5,200 \times g$  for 15 min at 4°C (Allegra X-30R, Beckman Coulter, Brea, CA). The supernatant was discarded and pellets were resuspended in 18 mL buffered peptone water (BPW; BD Difco, Franklin Lakes, NJ). Resuspended inoculum tubes were combined in equal proportions to prepare an inoculum cocktail, which was diluted in 8.7 L BPW to achieve a starting titer of 7.0 log CFU/mL.

**Spinach preparation and inoculation.** Fresh, unwashed spinach (*Spinacia oleracea*) was purchased from a local wholesale produce supplier. Stems were trimmed and product was held at 4°C until inoculation. At inoculation, 1000 g of spinach was submerged in *Salmonella* inoculum within a biosafety cabinet. Spinach was mixed intermittently and held in inoculum for 30 min to facilitate pathogen attachment. Spinach was removed from the inoculum and placed on stainless steel trays with grate overlays in the biosafety cabinet for 30 min to facilitate drying.

Spinach was flipped to facilitate uniform drying and permitted to dry for an additional 30 min prior to wash treatment.

**Preparation of wash treatments.** Three gallons of each water washing treatment were prepared: a wash containing sodium bisulfate and peroxyacetic acid (SBS-PAA), a chlorine wash, and tap water. SBS-PAA was prepared by adding 4.6 mL of a commercial produce wash (Tsunami 100™, Ecolab, St. Paul, MN) containing 15.2% peroxyacetic acid and 56.78 g of sodium bisulfate (0.50% w/v target concentration of SBS). A target concentration of 80 ppm PAA was measured using a manufacturer's test kit (Ecolab, St. Paul, MN). Chlorine wash was prepared by adding 19.4 mL germicidal bleach (Clorox Professional Products Company, Oakland, CA). The pH was adjusted by adding 1 N HCl to achieve pH 6.5-7.0, and total chlorine was measured using a free chlorine meter (Hanna Instruments, Woonsocket, RI) with a target concentration of 150 ppm total chlorine.

**Application of wash treatments.** A total 250 g of inoculated, unwashed spinach was set aside as a control. For each wash treatment, 250 g of inoculated spinach was placed in slotted containers (8.6 cm × 24.1 cm × 19 cm; InterDesign, Solon, OH) and submerged with gentle agitation in the wash treatment for 2 min. Samples subjected to chlorine wash were submerged in a subsequent tap water rinse with gentle agitation for 10 s following treatment to remove residual chlorine from the product. Washed, inoculated product was spun dry (centrifuged) in a salad spinner (26 cm diameter; Prepworks®, Kent, Washington) inside the biosafety cabinet to remove excess liquid. Pulling the salad spinner cord ten times standardized this process. Following centrifugation, samples (including control) were immediately sampled for pathogen enumeration and then packaged in retail display packages (26.4 × 16.6 cm; 50 g spinach) specifically designed for fresh-cut spinach (American Packaging Corporation, Columbus, WI; structure: 100

ga. OPP/70 ga. OPP; roll width: 18.875"; 4 lanes of continuous perforations with perforations spaced 0.30" apart; perforation flow rate:  $50 \pm 10$  sccm) and stored at 7°C to mimic retail storage conditions (O'Beirne, Gomez-Lopez et al. 2015). The ratio of spinach weight to packaging size was chosen to mimic what is used commercially.

**Salmonella enumeration.** *Salmonella* spp. populations were enumerated on day 0 and throughout shelf life (d 1, 3, 5, and 10). Briefly, 25 g of product was homogenized (Stomacher® 400 Circulator, Seward, Bohemia, NY) with 225 mL of Dey-Engley Neutralizing Broth (DNB; BD BBL™, New Jersey) for 60 s at 230 rpm. Samples were serially diluted in 0.1% peptone water (PW; BD Difco, Franklin Lakes, NJ) and spread plated onto xylose lysine tergitol-4 agar (XLT-4; Remel, Lenexa, KS) with and without a tryptic soy agar (TSA; BD Difco, Franklin, Lakes, NJ) overlay to recover injured cells, and incubated at 37°C for 24 h. The thin agar layer method (TAL) (Wu 2008) was used to enumerate injured cells by spread plating on XLT-4 overlaid with TSA.

**Statistical analysis.** Three replications were completed and data were analyzed using the MIXED procedure of Statistical Analysis Software (SAS 9.4; Cary, NC) to determine statistical significance ( $p \leq 0.05$ ) of the main effects (treatment, day) and interaction (treatment  $\times$  day).

## 4.4. RESULTS

**Sanitizer efficacy from injury-recovery media enumeration.** Enumeration on XLT-4 + TSA demonstrated treatment as a significant variable ( $P < 0.0001$ ). All treatments were significantly different from each other (Figure 4.1). The largest population of *Salmonella* (6.66 log CFU/g) was recovered from control (inoculated, unwashed) spinach. SBS-PAA treatment resulted in the largest (1.78 log CFU/g) and most significant ( $p < 0.00001$ ) reduction compared to

control. SBS-PAA was significantly more effective at reducing *Salmonella* populations than water ( $p < 0.001$ ) and chlorine ( $p < 0.0270$ ).

Sampling day ( $p = 0.0008$ ) was also a significant variable (Figure 4.2). *Salmonella* population variability was observed throughout shelf-life, with the largest population recovered on day 3 (6.20 log CFU/g), a statistically significant increase ( $p = 0.0003$ ) of 0.88 log CFU/g between days 0 and 3. In comparison to day 3, a significant decline in *Salmonella* populations was observed on days 5 (0.83 log CFU/g;  $p = 0.0005$ ) and 10 (0.53 log CFU/g;  $p = 0.0219$ ). *Salmonella* populations at the end of 10-days were statistically the same as on day 0 ( $p = 0.1256$ ).

**Sanitizer efficacy from selective media enumeration.** Treatment ( $p < 0.0001$ ) was the only significant variable (Figure 4.3). Control (inoculated, unwashed) spinach harbored the largest population of *Salmonella* (6.39 log CFU/g). While treating spinach with SBS-PAA resulted in the largest reduction (1.67 log CFU/g) in *Salmonella* compared to control, the reduction achieved by SBS-PAA was statistically the same ( $p = 0.2603$ ) as chlorine. Sampling day was not statistically significant ( $p = 0.1231$ ) when spinach populations were determined by plating onto XLT-4.

## 4.5. DISCUSSION

**The media discrepancy.** Subjecting inoculated samples to interventions is intended to reduce pathogen populations by killing the bacterial cells. It is possible, however, that these interventions result in cell injury rather than cell death for a portion of the population (Wu 2008), which can lead to challenges when enumerating populations. Because the spinach is a live, active product that is grown outside, it is possible that background flora are present prior to inoculation. Thus, the use of a selective media (in this case, XLT-4) to identify the pathogen-of-concern (*Salmonella*) is required. However, the presence of various chemicals, inhibitory chemicals, et

cetera in selective media creates an environment that may be too harsh for injured cells to grow (Wu 2008). The resultant enumeration may not be an accurate representation of the surviving *Salmonella* population. To address this issue, samples were plated on both the selective medium (XLT-4) and an injury recovery medium (XLT-4 plus TSA overlay). TSA is a non-selective medium that provides a favorable environment for the growth of injured cells (Wu 2008).

**Use of sodium bisulfate to destroy pathogens.** The use of sodium bisulfate has been explored as a pre-harvest intervention to control *Salmonella* in poultry. Adding SBS in an anaerobic *in vitro* chicken cecum model inhibited *Salmonella* Typhimurium growth, and the use of feed-grade SBS has been proposed as a pre-harvest control method for poultry operations (Rubinelli, Kim et al. 2017). Application of SBS to rendered protein meal (and not in aqueous form) failed to prevent *Salmonella* contamination (Cochrane, Huss et al. 2016). To our knowledge, this study represents one of the first experiments examining the use of SBS as part of a produce wash, and one of the first using a combined, synergistic treatment with PAA.

**Destroying *Salmonella* on spinach using combined treatments.** When Neal et al. (Neal, Marquez-Gonzalez et al. 2012) subjected *Salmonella* spp. inoculated spinach to 2% L-lactic acid, a 2.7 log CFU/g reduction was achieved; however, this treatment was applied at 55°C (Neal, Marquez-Gonzalez et al. 2012). In the same study, application of calcium hypochlorite (200 mg/L), peroxyacetic acid (80 mg/L), and chlorine dioxide gas resulted in reductions < 1.0 log CFU/g (Neal, Marquez-Gonzalez et al. 2012). In the present study, SBS-PAA surpassed the efficacy of 80 mg/L of PAA, by significantly reducing *Salmonella* populations by 1.67 and 1.78 log CFU/g when plated on XLT-4 and XLT-4+TSA, respectively.

Previously published studies report increased antimicrobial efficacy when interventions are used in combination. A combination of malic acid and lactic acid electrostatically sprayed on

*S. Typhimurium*-inoculated spinach achieved approximately 3.6 log CFU/disk reductions and disrupted biofilm formations as measured by crystal violet assay (Almasoud, Hettiarachchy et al. 2015). A combined treatment that primed spinach surfaces with nano-aerosolized water followed by H<sub>2</sub>O<sub>2</sub> dipping reduced *Salmonella* populations by 5.1 log CFU/g (Zhang, Oh et al. 2015). Another combined treatment, which was performed on *E. coli*-inoculated baby spinach, involved spraying Pro-San L (0.66% citric acid and 0.036% sodium lauryl sulfate) followed by vacuum cooling and ozonation under pressure, which reduced bacterial populations by 3.9 log CFU/g (Pyatkovskyy, Shynkaryk et al. 2017).

Despite efforts to prevent and/or minimize contamination of produce in the field and to prevent cross-contamination in processing and handling, continued outbreaks demonstrate an ongoing need for other non-chemical interventions (Murray, Wu et al. 2017). While non-chemical approaches, such as X-ray (Mahmoud, Bachman et al. 2010) and electron beam (e-beam) radiation (Neal, Cabrera-Diaz et al. 2008) have proven effective at reducing populations of *Salmonella* and other bacteria on spinach, the physical and economic costs may, like other non-chemical interventions, continue to be a barrier to implementation. It is likely, then, that the research presented herein may be more advantageous and relevant to industry.

**General conclusions.** Consumer studies have shown that purchasers of fresh produce are willing to pay a premium if the likelihood of foodborne illness is reduced 50% (Yu, Neal et al. 2018). Thus, research into effective chemical washes, like the SBS-PAA explored here, is warranted. Chlorine, an effective chemical at reducing microbial populations in plant systems (Beuchat, Ward et al. 2001), is frequently used by the produce industry in water washes; however, increasing numbers of outbreaks have challenged its efficacy (Ölmez and Kretzschmar 2009). Further, the environmental and health risks of chlorine have prompted producers to look

elsewhere (Gil, Selma et al. 2009, Ölmez and Kretzschmar 2009), especially when considering the generation of disinfection by-products that can cause health problems (Lee, Huang et al. 2018). The combination SBS-PAA wash was significantly more effective than chlorine at reducing *Salmonella* populations on spinach, when enumerated on injury recovery medium. Therefore, washing spinach in SBS-PAA may alleviate concerns about chlorine while providing an effective way to reduce the microbial load of spinach at levels similar to, or better than, chlorine. Quality was not evaluated in this study, but no dramatic visual effects were observed.

This study provides initial insight into the efficacy of SBS-PAA at reducing *Salmonella* on fresh-cut spinach. Future research should aim to optimize SBS-PAA efficacy. Multiple concentrations of SBS-PAA should be considered, as well as multiple washing steps. Quality of SBS-PAA-treated spinach will also be an important consideration in future research studies.

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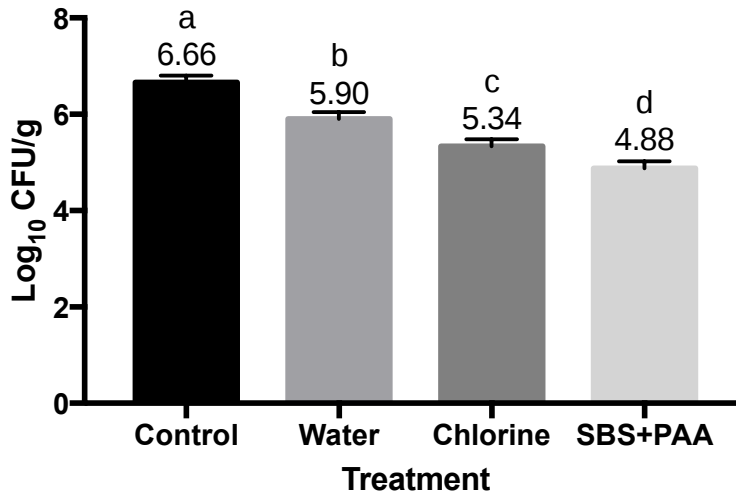
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## 4.7. FIGURES

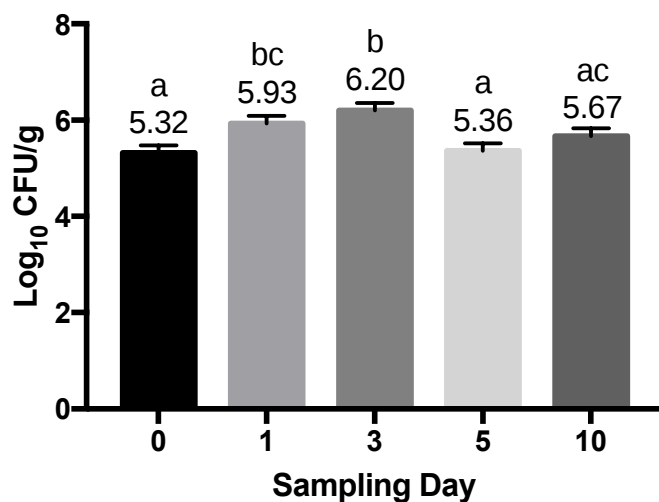
### *Salmonella* - XLT4 + TSA Injury Recovery Medium



**Figure 4.1:** Treatment significance for *Salmonella* populations on spinach subjected to postharvest washing and sampled throughout a ten day storage period (7°C) by plating on the injury recovery medium XLT-4 + TSA. Treatment ( $p < 0.0001$ ) was a significant variable and the treatment  $\times$  day interaction was not significant ( $p > 0.05$ ). Therefore, data are only shown by treatment.

<sup>a,b,c,d</sup> Denotes treatments that differ significantly ( $p \leq 0.05$ )

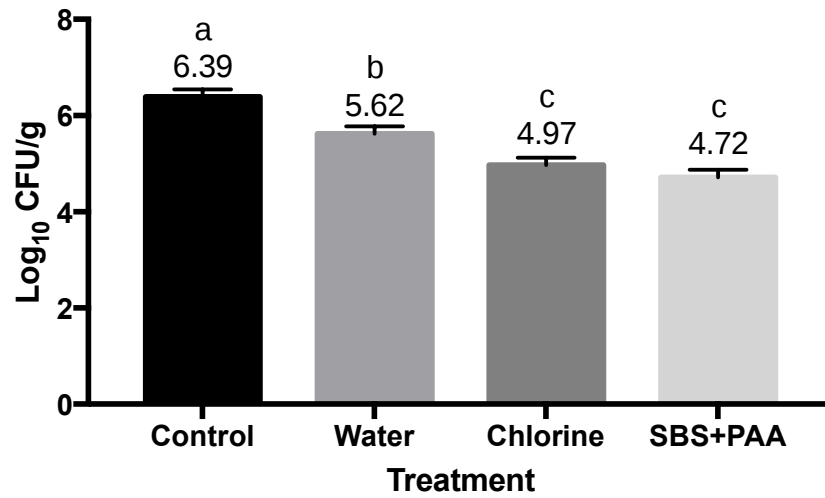
### ***Salmonella* - XLT4 + TSA Injury Recovery Medium**



**Figure 4.2: *Salmonella* populations by sampling day on spinach subjected to postharvest washing and sampled throughout a ten day storage period (7°C) by plating on the injury recovery medium XLT-4 + TSA. Day ( $p=0.0008$ ) was a significant variable and the treatment  $\times$  day interaction was not significant ( $p>0.05$ ). Therefore, data are only shown by day.**

<sup>a,b,c</sup> Denotes treatments that differ significantly ( $p\leq 0.05$ )

### ***Salmonella* - XLT4 Selective Medium**



**Figure 4.3: Selective media *Salmonella* populations on spinach subjected to postharvest washing and sample throughout a ten day storage period (7°C) by plating on XLT-4. Treatment ( $p < 0.0001$ ) was a significant variable and the treatment  $\times$  day interaction was not significant ( $p > 0.05$ ). Therefore, data are only shown by treatment.**

<sup>a,b,c</sup> Denotes treatments that differ significantly ( $p \leq 0.05$ )

## PART TWO: POLICY

### Chapter 5 - Literature Review

#### 5.1. NOTEWORTHY LAWS, REGULATIONS, AND GOVERNMENTAL INSTITUTIONS RELATED TO STEC AND SALMONELLA

##### 5.1.1. Introduction to U.S. food regulatory agencies and enabling acts.

Food law and regulation in the United States has a long and varied history.<sup>1</sup> Prior to 1906, food law was nested primarily in common law and secondarily (and increasingly) in a number of local, state, and federal ordinances, statutes, and laws; however, challenges related to interstate commerce (and thus, self-contained state laws) and self-represented litigation prohibited many consumers the ability to challenge food companies when products were defective. The first major federal laws passed relating to food safety were the 1906 *Pure Food and Drug Act* (PFDA) and the 1907 *Federal Meat Inspection Act* (FMIA). It was public outcry that led to the passage of PFDA and FMIA, an outcry emanating from the publication of Upton Sinclair's *The Jungle*, a 1904-1906 serial-based novel intended to alert the nation to the struggles facing America's working class. Instead, the novel shocked readers with its accounts of troubling food handling practices in Chicago slaughterhouses. Sinclair famously remarked, "I aimed at the public's heart, and by accident I hit it in the stomach."<sup>2</sup>

Prompted by *The Jungle*, as well as a scandal involving embalmed beef, Congress passed the PFDA as a means to control dangerous foods and drugs and curtail deceptive labeling

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<sup>1</sup> The author draws his own accumulated knowledge in this section, including notes given during *Food Law and Regulation*, a course taught by the author, guest lectures for foreign government officials (Cochran officials) taught by the author, author's publication in *Food Safety and Quality* magazine, as well as Sanchez, M. C. (2015), *Food Law and Regulation for Non-Lawyers: A U.S. Perspective*. Switzerland, Springer International Publishing, and Johnson, R. (2014) *The Federal Food Safety System: A Primer*. Washington, DC, Congressional Research Service.

<sup>2</sup> See: Eric Scholsser's introduction to *The Jungle*, Penguin Classics, Penguin Group (USA).

practices; however, PFDA lacked regulatory systems for premarket testing and reviews. After scores of children were poisoned by an antibiotic called Elixir of Sulfanilamide, Congress passed the 1938 *Federal Food Drug and Cosmetic Act* (FDCA), which is still the law today (the *FDA Food Safety Modernization Act* [FSMA] merely amended FDCA; other primary laws like the 2002 Bioterrorism Act and the 1947 Federal Insecticide, Fungicide, and Rodenticide Act [FIFRA]<sup>3</sup> serve as complements to the FDCA). Thus, by 1938, both the beef industry and the food industry at-large had their “enabling acts”: the 1938 FDCA and the 1907 FMIA. The passage of the 1957 *Poultry Products Inspection Act* (PPIA) and the 1970 *Egg Products Inspection Act* (EPIA) round out the four principal food laws in the United States. The four laws are codified at 21 United States Code (U.S.C.) § 301 *et seq.*, 21 U.S.C. § 601 *et seq.*, 21 U.S.C. § 451 *et seq.*, and 21 U.S.C. § 1031 *et seq.*, respectively.

Within the Executive Branch, food law is primarily enforced by the United States Department of Agriculture (USDA; via FMIA, PPIA, and EPIA) and the Food and Drug Administration (FDA; via FDCA).<sup>4</sup> Myriad other agencies (the Department of Homeland Security [DHS], the National Oceanic and Atmospheric Administration [NOAA], the Environmental Protection Agency [EPA], the Centers for Disease Control and Prevention [CDC], the Division of Alcohol Tobacco and Firearms [ATF], among others) are involved in ancillary roles. FDA oversees the safety of all domestic and imported food products not regulated by USDA. This is done through two main branches: the Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM). CFSAN oversees food safety research, enforcement, surveillance, state and local coordination, regulatory

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<sup>3</sup> Renée Johnson, *The Federal Food Safety System: A Primer* (Washington, DC: Congressional Research Service, 2014).

<sup>4</sup> FDA is part of the Department of Health and Human Services (HHS).

development, guidance document publication, and consumer safety information. CVM ensures animal feeds and drugs are free of residues and safe for the public.

USDA's food safety regulatory branch is the Food Safety and Inspection Service (FSIS), which regulates both the slaughter and processing of animal products as well as the safety and wholesomeness of meat and poultry sold for consumption. Authority extends to both domestic and foreign facilities. The bifurcated nature of food regulation between FDA and USDA creates some regulatory "headaches". For example, USDA regulates liquid, frozen, and dried eggs, and the laying facilities themselves, while FDA oversees shelled egg safety and the facilities that crack those eggs for cooking or ingredients. Most, but not all, seafood falls under FDA regulation; the exception, catfish, is inspected by USDA.<sup>5</sup> A facility producing frozen pizzas will have its pepperoni line continuously inspected by USDA, while cheese pizzas may only be inspected once every five years by FDA.

Both FDA and USDA rely on inspections to perform their duties to protect the food supply. USDA's aforementioned enabling acts permit the department continuous inspection authority over all regulated products. Thus, at slaughter all animals are inspected by FSIS personnel, and inspectors are always on-site when product processing occurs. FSIS can employ several regulatory enforcement mechanisms, including immediate regulatory control actions, the withholding of USDA stamps, the suspension of a facility, or the removal of inspectors.

Instead of continuous inspection, FDA conducts warrantless inspections of food facilities for cause or surveillance purposes. FDA's enforcement mechanisms include seizing or detaining product (requires court approval), recalls (now orderable by FDA with the passage of FSMA),

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<sup>5</sup> USDA-regulated catfish is a result of political jockeying and trade protection measures instituted by Congress. Namely, enhanced regulatory standards permit U.S.-raised catfish easier entry into the American market compared to catfish imported from Asian nations such as Vietnam, which struggle to meet the required standards. The author has enjoyed teaching about this unique food regulatory issue in *Food Law and Regulation*.

import refusals and import alerts, restraining orders or injunctions, and suspension of registration.<sup>6</sup>

### 5.1.2. Adulteration

While misbranding and other related labeling issues are certainly an important part of what FDA and USDA are mandated to do, perhaps the greatest duty of both agencies is to ensure that adulterated product does not reach the consumer. The prevention of adulteration is a primary policy concept explored in this dissertation, and thus adulteration merits discussion here. Each USDA enabling act, and the FDCA, have the same definition of adulteration, which reads, in part, that a product is adulterated...

- (1) If it bears or contains any poisonous or deleterious substance which may render it injurious to health...*
- (2) If it bears or contains any added poisonous or added deleterious substance...*
- (3) If it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food...*
- (4) If it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health...<sup>7</sup>*

Historically, there have been several economic reasons why a product may be adulterated; usually, this occurs when inferior ingredients are substituted for purported ingredients in a product. The primary concern with adulteration, however, is the presence of hazards, be they indirectly or directly a part of the product that renders it injurious to health, be it from a physical, chemical, or microbiological hazard or natural defect.

The 1993 *E. coli* O157:H7 Jack-in-the-Box outbreak, which sickened many and killed four, prompted USDA to shift its primarily organoleptic-based inspection methods to a hazards-

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<sup>6</sup> Registration of facilities was a new component passed with FSMA. All food producing facilities must register, and FDA can suspend that registration after a judicial hearing, effectively shutting down the facility.

<sup>7</sup> See: 21 U.S.C. §342(a), 21 U.S.C. § 453(g), 21 U.S.C. §601(m), and/or 21. U.S.C §1033(a).

based control system, and eventually (one year later) led to an unprecedented policy declaration related to adulteration. Specifically, FSIS administrator Michael R. Taylor declared *E. coli* O157:H7 an adulterant in a 1994 speech to the American Meat Institute Annual Convention.<sup>8</sup> Two years later, in 1996 USDA promulgated the Hazard Analysis Critical Control Point (HACCP)<sup>9</sup> systems regulation: this USDA rule encouraged food-hygiene related technological innovation and compelled companies to build upon good manufacturing practices (GMPs) and sanitation standard operating procedures (SSOPs) to create a system where hazards (chemical, physical, microbiological) are identified and control points for those hazards are established. Through critical limits, monitoring, corrective actions, verification, and recordkeeping, companies use HACCP to *anticipate* hazards instead of *responding* to them. The success of HACCP in the meat industry accompanied HACCP's implementation in the seafood and fruit juice industries as well. Similarly, the passage of FSMA included the requirement that FDA-regulated facilities have a system similar to HACCP for their facilities. Known as the Hazard Analysis Risk-Based Control Rule, or Preventative Controls Rule (HARPC), this rule revises FDA-related GMP regulations and introduces a mandatory hazard control system based on preventative controls.

Microbiologists and policymakers have debated whether the presence of certain bacteria causes a product to be *adulterated* under the accepted U.S. legal definitions mentioned above. Specific examples regarding Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* illustrate this debatable matter. Raw meat and poultry naturally carry a wide range of pathogens, which forces regulators to decide whether or not the presence of these pathogens, and

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<sup>8</sup> Michael R. Taylor, "Change and Opportunity: Harnessing Innovation to Improve the Safety of the Food Supply," *American Meat Institute Annual Convention* (1994).

<sup>9</sup> USDA Food Safety and Inspection Service, "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems," *Federal Register* 61, no. 144 (1996).

(significantly) the capability of the food preparer (*e.g.*, a chef, a backyard BBQ host, or a foodservice cook) to eliminate them, warrants adulterant status. Adulterant status for *E. coli* O157:H7 was established by the aforementioned Michael Taylor speech in 1994.<sup>10</sup> A decade and a half later, in 2011, following accumulated prevalence data, via a formal *Federal Register* announcement, other STEC serogroups (colloquially known as “The Big Six”: O26, O45, O103, O111, O121, and O145) were added as adulterants.<sup>11</sup> Specifically, these strains of *E. coli* can cause acute symptoms, severe sequelae, and death, and thus are considered adulterants in raw nonintact and ground beef. Thus, if *E. coli* O157:H7, or any of the non-O157 Big Six STEC, is found in these products, they are considered adulterated.

*Salmonella*, on the other hand, is not considered an adulterant, even though it has a high prevalence in outbreaks and is frequently found on raw product and in facilities. Why is there a discrepancy in the adulterant status between STECs and *Salmonella*? The discrepancy is rooted in case law, policy development, and food microbiology itself. First, USDA has court-based precedence for prohibiting adulterant status of *Salmonella*. In *American Public Health v. Butz* (1974), the Courts ruled that action to warn consumers that the beef product they were purchasing contained *Salmonella* was unnecessary because “American housewives and cooks normally are not ignorant or stupid and their methods of preparing and cooking of food do not ordinarily result in salmonellosis.”<sup>12</sup> In *Supreme Beef v. USDA* (2001) the Courts ruled that using *Salmonella* performance standards to shut down a beef plant was illegal because “*Salmonella*, present in a substantial proportion of meat and poultry products, is not an adulterant *per se*...because normal cooking practices...destroy the *Salmonella* organism, and therefore the

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<sup>10</sup> Taylor.

<sup>11</sup> USDA Food Safety and Inspection Service, "Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products," *Federal Register* 76 (2011).

<sup>12</sup> *American Public Health Association v. Earl Butz*, 73 1142 (1974).

presence of *Salmonella* in meat products does not render them ‘injurious to health’ for purposes of” the adulteration definition.<sup>13</sup>

The Center for Science in the Public Interest (CSPI) has argued and petitioned USDA to make, in the least, antibiotic-resistant (ABR) *Salmonella* an adulterant, claiming that the risk is analogous to STEC. Again, USDA has resisted. First, FSIS states that ABR *Salmonella* in beef or poultry products is not analogous to the presence of STEC in raw ground beef. Specifically, both the Big Six STEC and *E. coli* O157 have low infectious doses, can cause hemorrhagic colitis, and can lead to hemolytic uremic syndrome (HUS), health issues beyond the risks posed by ABR *Salmonella*.<sup>14</sup> Second, even though FSIS recommends ground beef be cooked to 160°F, USDA is aware that some consumers do not ordinarily cook to that temperature and thus leave them exposed to potentially deadly STEC. Meanwhile, poultry is cooked to proper temperatures consistently. Third, FSIS reaffirms that ordinary cooking of ground beef and poultry controls for *Salmonella*, and current scientific literature doesn’t support any difference between ABR and non-ABR *Salmonella* in terms of thermal resistance or other phenotypic attributes.<sup>15</sup> Fourth, the infectious dose of *Salmonella*, ABR or otherwise, is higher than STEC, and there are myriad factors in the food matrix that impact the infectious dose of *Salmonella*. Fifth, there is not conclusive evidence that ABR *Salmonella* is more virulent than susceptible *Salmonella*. Thus, USDA rests its denial on the fact that, based on current scientific literature, ABR *Salmonella* cannot be separated from susceptible *Salmonella* (not enough differences exist), and the characteristics of *Salmonella* “generally” fail to reach the level of criticality attained by STEC.

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<sup>13</sup> *Supreme Beef Processors v. United States Department of Agriculture*, 00 11008 (2001).

<sup>14</sup> "Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products."

<sup>15</sup> "HACCP Plan Reassessment for Not-Ready-to-Eat Comminuted Poultry Products and Related Agency Verification Procedures," *Federal Register* 77 (2012).

Processed meat products, some of which likely include hamburger, must undergo certain performance standards established by USDA-FSIS. In finished meat products, lethality performance standards for ready-to-eat (RTE) products require a 6.5-log reduction of *Salmonella* throughout the finished product (also codified at 9 Code of Federal Regulations [CFR] § 301, 317, 318, 320, and 381).<sup>16</sup> Similarly, methods to control *C. perfringens* and *C. botulinum* are also included in the performance standards. These stabilization standards require that a product's maximum internal temperature not remain between 130°F and 80°F for more than 1.5 hours nor between 80°F and 40°F for more than five hours.<sup>17</sup> Colloquially, the lethality performance standards are known as “Appendix A” and stabilization performance standards are known as “Appendix B.”

For producers of uncured meat patties (a common route for ground beef usage), further guidelines have been established in 9 CFR § 318.23. Namely, patties that are heat-processed must undergo permitted heat-processing temperature and time combinations. Patties that are partially-cooked or “char-marked” patties must bear labeling warning customers that the product is uncooked and must reach 160°F. Stabilization protocols similar to those in Appendix B are also spelled out in the regulation.<sup>18</sup>

### **5.1.3. The persistence of STEC and *Salmonella* foodborne illness outbreaks**

Despite such policy developments as the 1996 HACCP Rule and the STEC adulterant declarations, these pathogens and *Salmonella* continue to be major public health threats. Tables 5-1 and 5-2 below illustrate this.

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<sup>16</sup> "Performance Standards for the Production of Certain Meat and Poultry Products," *Federal Register* 64 (1999).

<sup>17</sup> *Ibid.*

<sup>18</sup> *Ibid.*

**Table 5-1: STEC outbreaks reported to CDC, 2008-2017<sup>19</sup>**

| <b>Year</b> | <b>Product</b>                     | <b>Strain</b> | <b>Cases (hospitalizations, HUS, deaths)</b>   |
|-------------|------------------------------------|---------------|------------------------------------------------|
| 2008        | Ground beef                        | O157:H7       | 49 (27, 1, 0)                                  |
| 2009        | Pre-packaged Cookie Dough          | O157:H7       | 72 (34, 10, 0)                                 |
| 2009        | Beef Products                      | O157:H7       | 23 (12, 2, 0)                                  |
| 2009        | Ground beef                        | O157:H7       | 26 (19, 5, 2)                                  |
| 2010        | Ground beef                        | O157:H7       | 21 (9, 1, 0)                                   |
| 2010        | Shredded Romaine Lettuce           | O145          | 30 (12, 3, 0)                                  |
| 2010        | Cheese                             | O157:H7       | 38 (15, 1, 0)                                  |
| 2011        | In-shell Hazelnuts                 | O157:H7       | 8 (4, 0, 0)                                    |
| 2011        | Lebanon Bologna                    | O157:H7       | 14 (3, 0, 0)                                   |
| 2011        | Travel to Germany/ Fenugreek Seeds | O104:H4       | Total: 2987 (unk., 855, 53)<br>US: 6 (4, 4, 1) |
| 2011        | Romaine Lettuce                    | O157:H7       | 58 (33, 3, 0)                                  |
| 2012        | Raw Clover Sprouts                 | O26           | 29 (7, 0, 0)                                   |
| 2012        | Unknown Source                     | O145          | 18 (4, 0, 1)                                   |
| 2012        | Organic Spinach & Spring Mix Blend | O157:H7       | 33 (13, 2, 0)                                  |
| 2013        | Frozen Food Products               | O121          | 35 (9, 2, 0)                                   |
| 2013        | Ready-to-Eat Salads                | O157:H7       | 33 (7, 2, 0)                                   |
| 2014        | Ground Beef                        | O157:H7       | 12 (7, 0, 0)                                   |
| 2014        | Raw Clover Sprouts                 | O121          | 19 (7, 0, 0)                                   |
| 2015        | Chipotle Mexican Grill             | O26           | 60 (22, 0, 0)                                  |
| 2015        | Rotisserie Chicken Salas           | O157:H7       | 19 (5, 2, 0)                                   |
| 2016        | Alfalfa Sprouts                    | O157:H7       | 11 (2, 0, 0)                                   |
| 2016        | Flour                              | O26 & O121    | 63 (17, 1, 0)                                  |
| 2016        | Beef, Veal, & Bison                | O157:H7       | 11 (7, 1, 0)                                   |
| 2017        | SoyNut Butter                      | O157:H7       | 32 (12, 9, 0)                                  |
| 2017        | Leafy Greens                       | O157:H7       | 25 (9, 2, 1)                                   |

<sup>19</sup> Centers for Disease Control and Prevention, "Reports of Selected *E. coli* Outbreak Investigations," U.S. Department of Health and Human Services <https://www.cdc.gov/ecoli/outbreaks.html>.

**Table 5-2: *Salmonella* outbreaks reported to CDC, 2013-2017<sup>20</sup>**

| <b>Year</b> | <b>Product</b>                       | <b>Strain (*multidrug resistant)</b>           | <b>Cases (hospitalizations, deaths)</b> |
|-------------|--------------------------------------|------------------------------------------------|-----------------------------------------|
| 2013        | Ground beef                          | Typhimurium                                    | 22 (7, 0)                               |
| 2013        | Chicken                              | Heidelberg                                     | 134 (33, 0)                             |
| 2013        | Cucumbers                            | Saintpaul                                      | 84 (17, 0)                              |
| 2013        | Tahini Sesame Paste                  | Montevideo & Mbandaka                          | 16 (1, 1)                               |
| 2013        | Chicken                              | Heidelberg*                                    | 634 (241, 0)                            |
| 2014        | Raw Cashew Cheese                    | Stanley                                        | 17 (3, 0)                               |
| 2014        | Chicken                              | Heidelberg                                     | 9 (2, 0)                                |
| 2014        | Organic Sprouted Chia Powder         | Newport, Hartford, & Oranienburg               | 31 (5, 0)                               |
| 2014        | Nut Butter                           | Braenderup                                     | 6 (1, 0)                                |
| 2014        | Bean Sprouts                         | Enteritidis                                    | 115 (29, 0)                             |
| 2014        | Cucumbers                            | Newport                                        | 275 (48, 1)                             |
| 2015        | Frozen Raw Tuna                      | Paratyphi & Weltevreden                        | 65 (11, 0)                              |
| 2015        | Raw, Frozen, Stuffed Chicken Entrees | Enteritidis*                                   | 15 (4, 0)                               |
| 2015        | Raw, Frozen, Stuffed Chicken Entrees | Enteritidis                                    | 5 (2, 0)                                |
| 2015        | Pork                                 | I 4,[5],12:i:-* & Infantis                     | 192 (30, 0)                             |
| 2015        | Cucumbers                            | Poona                                          | 907 (204, 6)                            |
| 2015        | Raw Sprouted Nut Butter              | Paratyphi                                      | 13 (0, 0)                               |
| 2016        | Organic Shake and Meal Products      | Virchow                                        | 33 (6, 0)                               |
| 2016        | Alfalfa Sprouts                      | Muenchen & Kentucky                            | 26 (8, 0)                               |
| 2016        | Pistachios                           | Montevideo & Senftenberg                       | 11 (2, 0)                               |
| 2016        | Alfalfa Sprouts                      | Reading & Abony                                | 36 (7, 0)                               |
| 2016        | Shell Eggs                           | Oranienberg                                    | 8 (2, 0)                                |
| 2017        | Maradol Papayas                      | Thompson, Kiambu, Agona, Gaminara, Senftenberg | 220 (68, 1)                             |
| 2017        | Maradol Papayas                      | Anatum                                         | 20 (5, 1)                               |

<sup>20</sup> "Reports of Selected *Salmonella* Outbreak Investigations," U.S. Department of Health and Human Services <https://www.cdc.gov/salmonella/outbreaks.html>.

|      |                 |                    |          |
|------|-----------------|--------------------|----------|
| 2017 | Maradol Papayas | Newport & Infantis | 4 (2, 0) |
| 2017 | Maradol Papayas | Urbana             | 7 (4, 0) |

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#### 5.1.4 Surveillance and food safety regulation.

An important facet of the food regulatory system is the ability to surveil microbiological behavior and metrics across the country. Doing so provides regulators hard data to assess whether policy-based interventions are working and clues them in to emerging issues. Microbial surveillance is “the systematic collection, analysis, and interpretation of data essential to the planning, implementation, and evaluation of public health practice.”<sup>21</sup> Numerous surveillance systems exist in the United States, most of which involve the tracking of foodborne illness by local, state, and national agencies. Nationally, this is principally accomplished by the CDC, working alongside other agencies such as FDA and USDA.<sup>22</sup>

CDC uses several surveillance systems to track foodborne illnesses. Often reliant upon data from state and local health agencies, the current surveillance systems include: the Foodborne Disease Active Surveillance Network (FoodNet), the Foodborne Disease Outbreak Surveillance System (FDOSS), the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS), the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet), the National Outbreak Reporting System (NORS), the National Electronic Norovirus Outbreak Network (CaliciNet), the Foodborne Disease Centers for Outbreak Response Enhancement (FoodCORE), the National Environmental Assessment Reporting System (NEARS), and the National Notifiable Diseases Surveillance Systems

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<sup>21</sup> World Health Organization, "Surveillance to Detect Foodborne Disease Outbreaks.", p. 9.

<sup>22</sup> Centers for Disease Control and Prevention, "Key Players in Foodborne Outbreak Response," <https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/key-players.html>.

(NNDSS).<sup>23,24</sup> These systems help identify community-level outbreaks that are more difficult to detect compared to those that occur in specific populations, such as in schools or hospitals.<sup>25</sup> Collaboration and consultation with food industry experts is important: production practices, consumer purchasing patterns (*e.g.*, shoppers' cards), and supply chain information are all valuable sources of information.<sup>26</sup>

**FoodNet** "conducts active surveillance for *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *Escherichia coli* O157 and non-O157 [serogroups], *Shigella*, *Vibrio*, and *Yersinia* infection diagnoses by laboratory testing of samples from patients."<sup>27</sup> It also tracks incidence of these infections over time and determines outbreak sources.<sup>28</sup> Doing so involves a cadre of clinical laboratories in ten states, surveys of physicians and the general population, and epidemiological studies.<sup>29</sup> FoodNet has, through case-control epidemiological studies, determined risk factors for sporadic enteric infections and developed a research platform to address emerging issues.<sup>30</sup> FoodNet reports are published in CDC's *Morbidity and Mortality Weekly Report* (MMRW), and other studies and reports are published online.<sup>31</sup>

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<sup>23</sup>Michael P. Doyle et al., "The Food Industry's Current and Future Role in Preventing Microbial Foodborne Illness within the United States," *Clinical Infectious Diseases* 61, no. 2 (2015).

<sup>24</sup>Centers for Disease Control and Prevention, "Surveillance & Data Systems," <https://www.cdc.gov/ncezid/dfwed/keyprograms/surveillance.html>.

<sup>25</sup>World Health Organization.

<sup>26</sup>Centers for Disease Control and Prevention, "Partnerships That Help Early in Foodborne Outbreak Investigations," <https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/partnerships.html>.

<sup>27</sup>"About FoodNet," <https://www.cdc.gov/foodnet/about.html>.

<sup>28</sup>Olga L. Henao et al., "Foodborne Diseases Active Surveillance Network—2 Decades of Achievements, 1996–2015," *Emerging Infectious Diseases* 21, no. 9 (2015).

<sup>29</sup>Centers for Disease Control and Prevention, "About FoodNet".

<sup>30</sup>Henao et al.

<sup>31</sup>Centers for Disease Control and Prevention, "Foodborne Illness Surveillance Systems," [https://www.cdc.gov/foodborneburden/pdfs/factsheet\\_g\\_surveillance.pdf](https://www.cdc.gov/foodborneburden/pdfs/factsheet_g_surveillance.pdf).

**FDOSS** collects data from state and local health agencies and integrates it into the Foodborne Outbreak Online Database (FOOD Tool), a web-based, searchable system.<sup>32</sup> This publicly-available database is frequently used by advocacy groups, the food industry, and the medical community. The annual data summaries it generates have been used in benchmarking the Healthy People 2020 food safety goals and other public health endeavors.<sup>33</sup>

**PulseNet** is a network of 83 U.S. laboratories that identifies foodborne illness outbreaks by comparison of pulsed-field gel electrophoresis (PFGE) DNA fingerprints. Using PFGE, to persons who consumed the same contaminated food are identified, which assists epidemiologists and public health officials in source attribution.<sup>34</sup> PulseNet permits quicker identification of illnesses that are truly part of an outbreak, and the interconnectedness of the program can detect outbreaks on a national scale.<sup>35</sup> PulseNet “has revolutionized the detection and investigation of foodborne disease outbreaks.”<sup>36</sup>

**NORS**, established in 2009, is an enteric disease reporting system that identifies outbreaks caused by bacterial, viral, parasitic, chemical, toxin, and unknown agents.<sup>37</sup> NORS integrates CDC reporting systems and approaches enteric disease from a *one health* perspective.<sup>38</sup> NORS data provides insight and data on food sources, settings correspondent to an outbreak, and other contributing factors.<sup>39</sup> The foodborne illness-related data from NORS is fed into the FOOD Tool online database.

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<sup>32</sup> "Surveillance for Foodborne Disease Outbreaks," <https://www.cdc.gov/foodsafety/fdoss/surveillance/index.html>.

<sup>33</sup> Ibid.

<sup>34</sup> "What Is PulseNet?," <https://www.cdc.gov/pulsenet/about/faq.html>.

<sup>35</sup> Ibid.

<sup>36</sup> "Foodborne Illness Surveillance Systems".

<sup>37</sup> "About NORS," <https://www.cdc.gov/nors/about.html>.

<sup>38</sup> The One Health approach advocated here is rooted in history. The author spent some time in 2017 at the National Library of Medicine researching the writings of John Shaw Billings, an early advocate of what is known as “One Health” today. The One Health approach was also advocated by Kansan and public health leader Samuel Crumbine who stated, “The health of each one of us depends on the health of all of us.”

<sup>39</sup> Centers for Disease Control and Prevention, "Foodborne Illness Surveillance Systems".

**CaliciNet** is CDC's norovirus outbreak surveillance network of federal, state, and local public health laboratories.<sup>40</sup> Norovirus is the most common cause of foodborne illness, accounting for 58% of illnesses in the most recent CDC study.<sup>41</sup> Both laboratory data (*i.e.*, genetic sequences of norovirus strains) and epidemiological data from norovirus outbreaks are electronically uploaded to the CDC database to help "link outbreaks to a common source, monitor norovirus strains that are circulating, and identify newly emerging norovirus strains."<sup>42</sup> CaliciNet is linked with NORS, as well.<sup>43</sup>

**FoodCORE** is similar to FoodNet in that it is a group of ten state and local health departments that blend laboratory, epidemiological, and environmental health teams to enhance surveillance, conduct better epidemiological investigations, and develop best practices for detection, investigation, response, and control.<sup>44</sup> Outbreaks caused by, STEC, *Salmonella* spp., and *Listeria monocytogenes* are monitored through FoodCORE.<sup>45</sup>

Finally, **NEARS** (the National Environmental Assessment Reporting System) is a surveillance system in which local public health agencies assist CDC in identifying the foods that cause outbreaks and the extrinsic reasons why an outbreak occurred.<sup>46</sup> CDC uses NEARS data to create corrective actions to eliminate risks.<sup>47</sup> Recent NEARS data has demonstrated that multiple characteristics comprise contributing factors. Further, contributing factor identification occurs

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<sup>40</sup> "Reporting and Surveillance for Norovirus: CaliciNet," <https://www.cdc.gov/norovirus/reporting/calicinet/index.html>.

<sup>41</sup> Elaine Scallan et al., "Foodborne Illness Acquired in the United States—Major Pathogens," *Emerging Infectious Diseases* 17, no. 1 (2011).

<sup>42</sup> Centers for Disease Control and Prevention, "Reporting and Surveillance for Norovirus: CaliciNet".

<sup>43</sup> "Foodborne Illness Surveillance Systems".

<sup>44</sup> "About FoodCORE," <https://www.cdc.gov/foodcore/about.html>.

<sup>45</sup> Ibid.

<sup>46</sup> "National Environment Assessment Reporting System (NEARS)," <https://www.cdc.gov/nceh/ehs/nears/index.htm>.

<sup>47</sup> Ibid.

more frequently when the etiologic agent is known.<sup>48</sup> The National Notifiable Diseases Surveillance System (*NNDSS*) collects notifiable disease case incidences from health providers for passive surveillance by CDC. Several foodborne diseases are reportable, including hemolytic uremic syndrome, listeriosis, and vibriosis, for example.<sup>49</sup> NARMS, another CDC initiative, was discussed in the dissertation's Part One's literature review (*i.e.*, chapter 1).

There are global foodborne illness tracking initiatives as well. PulseNet International is a consortium of 88 countries in seven global regions performing PFGE and transitioning to whole genome sequencing (WGS) to better identify foodborne illness outbreaks.<sup>50</sup> A coordinating laboratory in each region conducts "training and quality control, quality assurance programs, and [organization of] regular conference calls, meetings, and communication of epidemiological information."<sup>51</sup> The World Health Organization (WHO) is also involved in foodborne epidemiological training through the WHO Global Foodborne Infections Network (GFN), "a global effort to build capacity to detect, control, and prevent foodborne and other enteric infections from farm to table."<sup>52</sup> For example, labs in the GFN track the fifteen most frequently isolated *Salmonella* serovars and submit descriptive analyses regarding strains to an internet database.<sup>53</sup>

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<sup>48</sup> L. G. Brown et al., "Outbreak Characteristics Associated with Identification of Contributing Factors to Foodborne Illness Outbreaks," *Epidemiology and Infection* 145, no. 11 (2017).

<sup>49</sup> Centers for Disease Control and Prevention, "Foodborne Illness Surveillance Systems".

<sup>50</sup> "PulseNet International: On the Path to Implementing Whole Genome Sequencing for Foodborne Disease Surveillance," <https://www.cdc.gov/pulsenet/pdf/pulsenet-international-wgs.pdf>.

<sup>51</sup> Efrain M Ribot and Kelley B Hise, "Future Challenges for Tracking Foodborne Diseases," *PulseNet, a 20-year-old US surveillance system for foodborne diseases, is expanding both globally and technologically* (2016)., p. 1499.

<sup>52</sup> R. S. Hendriksen et al., "Global Monitoring of *Salmonella* Serovar Distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of Quality Assured Laboratories from 2001 to 2007," *Foodborne Pathog Dis* 8, no. 8 (2011)., p. 888.

<sup>53</sup> *Ibid.*

## 5.2. INTRODUCTION TO POLICY ANALYSIS AND POLICY DEVELOPMENT

### 5.2.1. Introduction.

To perform a policy analysis, several conceptual frameworks are available.<sup>54</sup> For this literature review, the eight-step policy analysis framework by Eugene Bardach has been adopted.<sup>55</sup> For illustration and application purposes, each step in Bardach's framework is explored in two separate food policy contexts: (1) FSMA and (2) intentional contamination of food.<sup>56</sup> FSMA is explored because the law was necessitated by several important factors and events, including notable outbreaks, consumer demands, and pressure placed upon lawmakers to correct perceived lapses in safety. The intentional contamination of a food product is explored because both *resilience* and *forecasting* can be effective tools in the battle against food contamination events, be they intentional or unintentional; these two concepts of *resilience* and *forecasting*—as well as the related phenomenon of “unknown unknowns” in food safety challenges—are explored later in this literature review and especially relate to intentional contamination events. The steps for a Bardach analysis are given in Table 5-3. In 5.2.2., these steps will be used to analyze a past policy development phenomenon (the passage of FSMA) as well as an ongoing policy dilemma (how to allocate resources to address the risk of an intentional contamination of the food supply).<sup>57</sup>

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<sup>54</sup> For example, *Policy Analysis: Concepts and Practice* (4<sup>th</sup> Ed.) by David L. Weimer and Aidan R. Vining (Pearson Education: Upper Saddle River, NJ, 2005) or Centers for Disease Control and Prevention, "CDC's Policy Analysis Framework," ed. U.S. Department of Health and Human Services (Atlanta, GA 2013).

<sup>55</sup> Eugene Bardach, *A Practical Guide for Policy Analysis: The Eightfold Path to More Effective Problem Solving* (New York, NY: Chatham House Publishers of Seven Bridges Press, LLC, 2000).

<sup>56</sup> To avoid excess citations and footnotes, from this point forward, Bardach will be cited only if direct quotations are used.

<sup>57</sup> Bardach references the “Taxi Driver Test” in which policies must be persuasive enough to convince a taxi driver (who is not an expert in the matter and who is not easily convinced) maneuvering through New York City (with attention and time limits).

**Table 5-3: Bardach’s Eightfold Path for Policy Analysis and Problem Solving**

| Step | Task                       | Action Items                                                                                                                                      |
|------|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | Define the problem         | Think of deficits and excesses; determine if intervention is necessary; quantify problems and opportunities                                       |
| 2    | Assemble some evidence     | Think extensively about the problem and collect data/information                                                                                  |
| 3    | Construct the alternatives | Model the system; design a new policy considering all dimensions; understand alternatives and the solution are not necessarily mutually exclusive |
| 4    | Select the criteria        | Ensure the process is efficient, fair, and practical                                                                                              |
| 5    | Project the outcomes       | Quantify if possible; confront the optimist problem                                                                                               |
| 6    | Confront the trade-offs    | Focusing on outcomes, find the policy that dominates on important outcomes                                                                        |
| 7    | Decide                     |                                                                                                                                                   |
| 8    | Tell your story            | Develop a narrative and gauge the audience’s reaction                                                                                             |

Adapted from Bardach, E. (2000). A Practical Guide for Policy Analysis: The Eightfold Path to More Effective Problem Solving. New York, NY, Chatham House Publishers of Seven Bridges Press, LLC.

## **5.2.2. Problems, evidence, and policymaking: Bardach’s framework.**

### **5.2.2.1. Define the Problem.**

***Problem definition in the run-up to FSMA.*** Beginning in the mid-1990s and extending into the 2000s, the sources of foodborne illness outbreaks shifted from animal protein-based products to previously unseen food vehicles, including peanut butter, frozen foods, spinach, and cantaloupe. The new outbreaks not only involved new products, they were increasingly publicized and scrutinized by the national media and rapidly shared across the newly-arriving,

nearly omnipresent tool of social media. Thus, politicians found it necessary to update, or modernize, food safety regulation in the United States.<sup>58</sup>

***Problem definition for intentional-contamination threats.*** The terrorism events of September 11, 2001 (9/11) compelled policymakers to reevaluate the safety of various American infrastructures. DHS must address security vulnerabilities that impact several sectors of America's economy, security, and general well-being. By its very nature, the food system, with its scope, size, and importance to daily life, displays such vulnerability. Some have stated that "the concern that bioterrorism will impact agriculture in rural America, namely, crops in the fields, hoofed animals, and food-safety issues in the food chain between the slaughterhouse and/or processing facilities and the consumer, has only grown."<sup>59</sup>

#### **5.2.2.2. Assemble Some Evidence.**

***Evidence used by advocates for FSMA.*** The two tables of STEC and *Salmonella* outbreaks (Tables 5-1 and 5-2) constitute the majority of evidence that was needed to compel lawmakers to pass FSMA.<sup>60</sup> These include, but are not limited to, such critical outbreaks as the melamine in pet food and infant formula scandal,<sup>61,62,63</sup> the 2006 *E. coli* O157:H7 spinach outbreak,<sup>64</sup> the 2009 *Salmonella* Typhimurium Peanut Corporation of America (PCA)

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<sup>58</sup> Daniel A. Unruh, Sara E. Gragg, and Justin J. Kastner, "The Politics of Food Safety Regulations," *Food Quality & Safety* (2016). This commentary, published online at <http://www.foodqualityandsafety.com/article/commentary-politics-food-safety-regulations/>, explores other historical and contemporary food safety policy developments.

<sup>59</sup> Jane A. Bullock, George D. Haddow, and Damon P. Coppola, "All-Hazards Emergency Response and Recovery," in *Introduction to Homeland Security, 5th Ed.* (Waltham, MA: Butterworth-Heinemann, 2016)., p. 430.

<sup>60</sup> Obviously, the outbreaks in the tables that transpired after 2011 were not part of the lawmakers' calculus.

<sup>61</sup> Christopher Bodeen, "China Executes 2 People over Tainted Milk Scandal," *The World Post* 2010.

<sup>62</sup> David Barboza and Alexi Barrionuevo, "Filler in Animal Feed Is Open Secret in China," *The New York Times*, April 30 2007.

<sup>63</sup> T. Alcorn and Y. Ouyang, "China's Invisible Burden of Foodborne Illness," *Lancet* 379 (2012).

<sup>64</sup> Umid M. Sharapov et al., "Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated with Consumption of Fresh Spinach: United States, 2006," *Journal of Food Protection* 79, no. 12 (2016).

outbreak,<sup>65</sup> the 2007 *Salmonella* Banquet pot pie outbreak,<sup>66</sup> the 2007 *E. coli* O157:H7 frozen beef patties outbreak,<sup>67</sup> the 2008 *Salmonella* Saintpaul outbreak in hot peppers,<sup>68</sup> and the 2009 *E. coli* O157:H7 Nestle Toll House cookie dough outbreak.<sup>69</sup>

The PCA *Salmonella* Typhimurium outbreak was a multistate outbreak that sickened 714 persons across 46 states occurring between September 1, 2008 and April 20, 2009.<sup>70,71</sup> Nine people died in the outbreak and 166 were hospitalized.<sup>72</sup> The implicated vehicles were peanut products produced at PCA's Blakely, Georgia, and Plainview, Texas, facilities.<sup>73,74</sup> The outbreak was a watershed event for several reasons. First, the production facilities had notable lapses in food safety infrastructure, including leaking roofs, evidence of animal feces and feathers near and on the product, presence of cockroaches, mold on ceilings and walls, storage of raw material near cooked material, and inadequate time × temperature cooking protocols.<sup>75,76</sup> Secondly, PCA would frequently re-test *Salmonella*-positive peanut butter samples until a negative result was obtained, and management regularly advocated workers "get around" certificates of analysis that were positive for *Salmonella*.<sup>77</sup> Finally, products were often shipped to vulnerable populations,

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<sup>65</sup> Elizabeth Cavallaro et al., "Salmonella Typhimurium Infections Associated with Peanut Products," *New England Journal of Medicine* 365, no. 7 (2011).

<sup>66</sup> Centers for Disease Control and Prevention, "Multistate Outbreak of *Salmonella* Infections Associated with Frozen Pot Pies --- United States, 2007," *Morbidity and Mortality Weekly Report* 57, no. 47 (2008).

<sup>67</sup> "Multistate Outbreak of *E. coli* O157 Infections Linked to Topp's Brand Ground Beef Patties (Final Update)," <https://www.cdc.gov/ecoli/2007/ground-beef-patties-10-26-2007.html>.

<sup>68</sup> Casey Barton Behraves et al., "2008 Outbreak of *Salmonella* Saintpaul Infections Associated with Raw Produce," *New England Journal of Medicine* 364, no. 10 (2011).

<sup>69</sup> Centers for Disease Control and Prevention, "Multistate Outbreak of *E. coli* O157:H7 Infections Linked to Eating Raw Refrigerated, Prepackaged Cookie Dough (Final Update)," <https://www.cdc.gov/ecoli/2009/cookie-dough-6-30-2009.html>.

<sup>70</sup> "Multistate Outbreak of *Salmonella* Typhimurium Infections Linked to Peanut Butter, 2008-2009 (Final Update)," <https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html>.

<sup>71</sup> Cavallaro et al.

<sup>72</sup> Ibid.

<sup>73</sup> Paul Leighton, "Mass *Salmonella* Poisoning by the Peanut Corporation of America: State-Corporate Crime Involving Food Safety," *Critical Criminology* 24, no. 1 (2016).

<sup>74</sup> Cavallaro et al.

<sup>75</sup> Leighton.

<sup>76</sup> Cavallaro et al.

<sup>77</sup> Leighton.

including schools and nursing homes (the young and elderly are more susceptible to *Salmonella* from an immunological perspective).<sup>78</sup> The PCA outbreak clearly demonstrated that a rogue corporation (*i.e.*, PCA) was not under enough regulation to properly protect the public, and thus, regulation (*i.e.*, FSMA) was necessary.<sup>79</sup> Of course, PCA presented several ethical dilemmas related to food regulation. For more on the role of ethics in food regulation, please see section 5.2.3.

Another nationwide *Salmonella* outbreak occurred in 2009 when 1500 persons (21% hospitalized; 2 deaths) were sickened with *Salmonella* Saintpaul following consumption of jalapeno and serrano peppers.<sup>80</sup> This outbreak is highlighted as an example for two reasons. First, the outbreak was a produce-related outbreak, confirming to FDA that products regulated by the agency likely deserved food safety scrutiny similar to the levels required for USDA-regulated animal products (put another way, there were more outbreaks occurring in non-animal protein products, and those items are FDA-regulated). Second, the outbreak caused high levels of externalities: when CDC and FDA incorrectly blamed tomatoes for the outbreak, consumers and companies stopped making tomato purchases, costing the industry upwards of \$100 million in Florida and \$14 million in Georgia alone.<sup>81</sup>

***Evidence justifying policy action for intentional contamination.*** Some have argued that the American food system is at risk because American agriculture is widespread (*e.g.*, large fields with lax security), concentrated (*e.g.*, cattle feeding operations contain many animals in a small area), and susceptible to various threat agents (*e.g.*, microorganisms and toxins that are

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<sup>78</sup> Ibid.

<sup>79</sup> Ibid.

<sup>80</sup> Barton Behravesh et al.

<sup>81</sup> Pew Charitable Trusts, "Breakdown: Lessons to Be Learned from the 2008 *Salmonella* Saintpaul Outbreak," [http://www.pewtrusts.org/~media/legacy/uploadedfiles/phg/content\\_level\\_pages/reports/psprptlessonssalmonella2008pdf.pdf](http://www.pewtrusts.org/~media/legacy/uploadedfiles/phg/content_level_pages/reports/psprptlessonssalmonella2008pdf.pdf). These two states were presumed to be the source of the unfairly accused tomatoes.

harmful to plants, animals, and/or humans).<sup>82,83</sup> Major foodborne illness outbreaks, while not intentional (*i.e.*, the result of lapses in HACCP or simply the result of ordinary microbiological contamination), provide justification for concern about the possibility for major intentional (perhaps, on a 9/11-sized scale) outbreaks.<sup>84</sup> Moreover, an intentional attack has already occurred on U.S. soil: in 1984, members of a cult devoted to the Bhagwan Shree Rajneesh intentionally contaminated salad bars with *Salmonella enterica* Typhimurium in order to sicken enough individuals to sway a local election in The Dalles, Oregon. Cult members were able to sicken 751 persons in the attack.<sup>85</sup> This event demonstrated how such an event could easily occur again. As Török et al. (1997) state:

*It seems unlikely that any regulation of commercially available pathogens could have prevented this outbreak. It would not be necessary to purchase them because this type of culture could be easily obtained from clinical isolates or from raw foods of animal origin available in grocery stores. Production of large quantities of bacteria is inexpensive and involves simple equipment and skills.*<sup>86</sup>

### 5.2.2.3. Construct the Alternatives.

***Alternatives to FSMA as a regulatory tool for food safety.*** When policymakers were debating the best policy directive to address increasing food safety challenges, they had the idea that was chosen (updating FDA by way of FSMA), and several other alternatives, including: (1) maintain the *status quo*; (2) pass a new food safety law that impacted (a) USDA, (b) USDA and

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<sup>82</sup> Peter Chalk, "Hitting America's Soft Underbelly: The Potential Threat of Deliberate Biological Attacks against the U.S. Agricultural and Food Industry," (Arlington, VA: RAND Corporation, 2004).

<sup>83</sup> Trevor Maynard, "Food System Shock," in *Emerging Risk Report - 2015 Innovation Series* (London, UK: Lloyd's of London, 2015).

<sup>84</sup> Shaun P. Kennedy and Frank P. Busta, "Biosecurity: Food Protection and Defense," in *Food Microbiology: Fundamentals and Frontiers*, ed. M.P. Doyle and L.R. Beuchat (Washington, D.C.: ASM Press, 2007).

<sup>85</sup> T. J. Török et al., "A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars," *JAMA* 278, no. 5 (1997). This event, and the Rajneeshee cult as a whole, are the subject of an upcoming Netflix documentary entitled *Wild Wild Country*.

<sup>86</sup> *Ibid.*, p. 394. Had the Rajneeshee used a more virulent pathogen, such as STEC or the typhoid-causing *Salmonella* Typhi, hundreds may have died.

FDA, or (c) an entirely new food safety agency; or (3) reduce funding and hope the free market tackled the issue.

***Alternatives to allocating resources to intentional contamination events.*** If the policy question is the benefit of allocating more resources to protecting the FA sector, the alternatives are (1) maintain the *status quo* or (2) decrease resource allocation.

#### **5.2.2.4. Select the Criteria.**

Both the passage of a new food safety law and the allocation of more resources to address the risk of an intentional adulteration of a food product should be evaluated using *efficiency*, *equality*, and *practicality*.<sup>87</sup>

***Criteria used by policymakers in the regulatory saga of FSMA.*** Issues of efficiency, equality, and practicality were invoked by not only the politicians putting forth bills that eventually led to FSMA, but also other federal policy research groups. A classic example of this is a report entitled “Overseeing the U.S. Food Supply: Steps Should Be Taken to Reduce Overlapping Inspections and Related Activities.”<sup>88</sup> The debates regarding the “Tester Amendment”, which advocated exemptions or delays in implementation for small-scale farmers and producers, also demonstrates the use of efficiency, equality, and practicality by lawmakers.<sup>89</sup>

***Suggested criteria to consider when regulating intentional adulteration.*** In terms of *efficiency*, more resource allocation to this problem would not be justified. Many systems in place to control for unintended hazards (*i.e.*, natural contamination) would control for an

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<sup>87</sup> Bardach., pp. 20-27.

<sup>88</sup> U.S. Government Accountability Office and Robert A. Robinson, "Overseeing the U.S. Food Supply: Steps Should Be Taken to Reduce Overlapping Inspections and Related Activities (GAO-05-549t)," (U.S. Government Accountability Office, 2005).

<sup>89</sup> Neva Hassanein, "Matters of Scale and the Politics of the Food Safety Modernization Act," *Agriculture and Human Values* 28, no. 4 (2011). The Tester amendment was named for Senator Jon Tester of Montana who, along with Senator Kay Hagan of North Carolina, advocated on behalf of small scale, organic, and niche food producers. Being from Montana, Tester had many persons in his state that qualified for these exemptions.

intentional contamination, unless a particularly virulent bacterium or toxin were used. But, the laboratory equipment and production knowledge is likely cost- and knowledge-prohibitive to terrorists. Regarding *equality*, budgets are already strained for agencies such as HHS and USDA, and forcing them to divert funds to something that is likely already controlled-for may hamper the execution of other programs and initiatives that deserve funding. For *practicality*, increased resource allocation is legally acceptable. However, political acceptability is difficult to forecast; it is easy to imagine that, on the surface, policymakers would desire programs that increase public safety, but if that funding comes from something more immediate (such as school funding, addressing the opiate epidemic, et cetera), then acceptability may decrease. Policies “should be robust enough so that even if the implementation process does not go very smoothly, the policy outcomes will still prove to be satisfactory.”<sup>90</sup> Increasing funding for intentional adulteration may not have that robustness.

#### **5.2.2.5. Project the Outcomes.**

***Outcomes achieved following the passage of FSMA.*** Selected rules promulgated to enforce the mandates of FSMA are given in table 5-4 below. The beneficial outcomes of the Preventative Controls rule and Produce Safety rule are explored in the next two paragraphs.

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<sup>90</sup> Bardach., p. 25.

**Table 5-4: Foundational FSMA rules<sup>92</sup>**

| <b>Rule</b>                                                                                                 | <b>Notice Date</b> | <b>Summary</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | <b>Citation</b>   |
|-------------------------------------------------------------------------------------------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| Accreditation of Third-Party Certification Bodies to Conduct Food Safety Audits and To Issue Certifications | 27 November 2015   | Accreditation of third-party certification bodies to conduct food safety audits of foreign food entities and to issue food and facility certifications. These certifications will be required for participation in the voluntary qualified importer program (VQIP). FDA expects that these regulations will increase efficiency by reducing the number of redundant food safety audits.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 80 FR 74569-74667 |
| Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food     | 17 September 2015  | Amends the regulation for Current Good Manufacturing Practice In Manufacturing, Packing, or Holding Human Food in two fundamental ways. First, it modernizes the long-standing current good manufacturing practice requirements. Second, it adds requirements for domestic and foreign facilities to establish and implement hazard analysis and risk-based preventive controls for human food. The rule is intended to build a food safety system for the future that makes modern, science- and risk-based preventive controls the norm across all sectors of the food system.                                                                                                                                                                                                                                                                                             | 80 FR 55907-56168 |
| Foreign Supplier Verification Programs for Importers of Food for Humans and Animals                         | 27 November 2015   | The regulation requires importers to verify that food they import into the United States is produced in compliance with the hazard analysis and risk-based preventive controls and standards for produce safety provisions of FDCA, is not adulterated, and is not misbranded with respect to food allergen labeling.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 80 FR 74225-74352 |
| Mitigation Strategies To Protect Food Against Intentional Adulteration                                      | 27 May 2016        | Requires facilities to address hazards that may be introduced with the intention to cause wide scale public health harm. These food facilities are required to conduct a vulnerability assessment to identify significant vulnerabilities and actionable process steps and implement mitigation strategies to significantly minimize or prevent significant vulnerabilities identified at actionable process steps in a food operation.                                                                                                                                                                                                                                                                                                                                                                                                                                      | 81 FR 34165-34223 |
| Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption                | 27 November 2015   | Establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of produce. Standards do not apply to produce that is rarely consumed raw, produce for personal or on-farm consumption, or produce that is not a raw agricultural commodity. In addition, produce that receives commercial processing that adequately reduces the presence of microorganisms of public health significance is eligible for exemption from the requirements of the rule. The rule sets forth procedures, processes, and practices that minimize the risk of serious adverse health consequences or death, including those reasonably necessary to prevent the introduction of known or reasonably foreseeable biological hazards into or onto produce and to provide reasonable assurances that the produce is not adulterated on account of such hazards. | 80 FR 74353-74568 |

<sup>92</sup> Food and Drug Administration, "FDA Food Safety Modernization Act (FSMA) Rules and Regulated Programs," <https://www.fda.gov/Food/GuidanceRegulation/FSMA/>.

The Preventative Controls rule not only updated current GMPs to a level commensurate with modern technology, it also compelled food companies to create the aforementioned HARP-C plans. Within these plans, food facilities identify physical, chemical, and microbiologically hazards. Then, facilities institute preventative controls for those hazards that require extra control, be it for process (*e.g.*, cooking, acidifying), food allergen, or sanitation controls. Once the preventative controls are established, they must be written, monitored, and corrected (and those corrections verified). Finally, facilities must implement a risk-based supply chain program to identify any hazards that may enter a facility through raw materials; further, facilities must implement a recall plan should a hazard enter commerce.<sup>93</sup>

The robust Produce Safety rule will affect several outcomes. First, water quality for crop application will be monitored closely, including testing of untreated water for indicators of fecal contamination. Standards for biological soil amendments, including raw manure application and stabilized compost application, have been established to control cross-contamination. New requirements to prevent contamination of sprouts, a product commonly implicated in foodborne illness outbreaks, have been included in the rule. New requirements for health and hygiene, including keeping ill workers out of the field, providing handwashing opportunities for workers, and training and education of workers and supervisors are now required. Finally, standards for equipment, tools, and buildings have been designed to prevent produce contamination, especially produce that is covered.<sup>94</sup>

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<sup>93</sup> "FSMA Final Rule for Preventative Controls for Human Food," <https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm>.

<sup>94</sup> "FSMA Final Rule on Produce Safety," <https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm>.

***Expected outcomes of intentional adulteration resource allocation.*** Allocating more resources to intentional food adulteration would increase scrutiny on an already highly-regulated industry. Meat, poultry, seafood, and fruit juice producers are already bound by the HACCP program, which creates vast amounts of paperwork and documentation (and, in turn, a level of oversight that would control for an adulteration unless an act of subterfuge impacted the program). FSMA's HARPC requirement, under the preventative controls rule, essentially requires HACCP control for non-HACCP regulated products.<sup>95</sup> Increasing oversight will add more paperwork and scrutiny to a system that has enough of both already. If anything, the value of resource allocation toward ensuring a safe food and agriculture sector has been mandated by policy. Under Presidential Policy Directive 21, food and agriculture is designated a critical infrastructure sector.<sup>96</sup> In some ways, ongoing resource allocation is already in-progress: Congress passed and the President recently signed an amendment to the 2002 *Homeland Security Act* to coordinate DHS efforts to protect food and agriculture through oversight and integration of programs and preparedness measures.<sup>97,98</sup>

#### **5.2.2.6. Confront the Trade-offs.**

***Challenges and trade-offs following the passage of FSMA.*** Numerous challenges presented themselves upon the passage of FSMA. The regulatory mandates would require many rules, which, in the context of rulemaking and administrative law, requires numerous *Federal Register* notices, opportunities for comment, comment responses, and final notice of the rules.

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<sup>95</sup> "FSMA Final Rule for Preventative Controls for Human Food".

<sup>96</sup> Barack Obama, "Presidential Policy Directive/PPD-21 -- Critical Infrastructure Security and Resilience," ed. Office of the Press Secretary (The White House: Washington, D.C.2015). Responsibility for managing this sector fall under the USDA, HHS, and DHS.

<sup>97</sup> Food Safety News Desk, "President Trump Likely to Sign Popular Agro-Terrorism Bill," Food Safety News, <http://www.foodsafetynews.com/2017/06/president-trump-likely-to-sign-popular-agro-terrorism-bill/#.Wblyr7KGOUk>.

<sup>98</sup> 115th Congress of the United States, "Securing Our Agriculture and Food Act," in *H.R. 1238* (Washington, D.C.: U.S. Government Publishing Office, 2017).

Rarely do new rules eliminate tasks and requirements: the norm is that more is expected of those that must follow the rule. Thus, an important trade-off to note is the monetary and time requirements imposed by new FSMA rules. This is coupled with the duration of time it took from passage to the issuance of new rules, a process that is ongoing at the time of this dissertation's writing. For a specific example, a survey of produce growers found that the recordkeeping, field inspection, and water testing requirements of the Produce Safety rule will present the greatest implementation challenge, particularly for small growers and sustainable growers.<sup>99</sup>

***Trade-offs associated with allocating resources to intentional contamination control.***

Ensuring “preparedness for a bioterrorist event affecting the food supply...entails augmentation of the traditional public-health infrastructure to enhance disease surveillance, accelerate capacity of laboratory detection, rapidly investigate and control outbreaks, and develop capacity for response to mass-casualty disasters.”<sup>100</sup> While such activity is important, it is being done within the mission and activity of DHS already on several fronts and through several other agencies, to protect the American food and agriculture. Not increasing resource allocation to food and agriculture to explicitly control for an intentional adulteration may, at first, seem like a nod towards relaxed standards (trade-offs), but in fact, the systems are already functioning under this control.

**5.2.2.7. Decide.**

***The FSMA example: did regulators make the right call?*** In many ways, FSMA and its attendant rules are still in their infancy. It is generally accepted that zero risk of acquiring a

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<sup>99</sup> Aaron Adalja and Erik Lichtenberg, "Implementation Challenges of the Food Safety Modernization Act: Evidence from a National Survey of Produce Growers," *Food Control* 89 (2018).

<sup>100</sup> Jeremy Sobel, Ali S Khan, and David L. Swerdlow, "Threat of a Biological Terrorist Attack on the US Food Supply: The CDC Perspective," *Lancet* 359 (2002), p. 874.

foodborne illness is unachievable; however, FSMA responsibly addresses this challenges by applying science- and risk-based preventative measures from farm to fork.<sup>101</sup> As Doyle et al. (2015) state:

*This new food safety system will undoubtedly have some unanticipated weak links during its early stages of implementation. However, over the long term, this new and improved food safety system should lead to a safer food supply and, in turn, to a reduced burden of foodborne illness.*<sup>102</sup>

The continued accumulation of environmental and product microbial testing results should persist to best identify any weaknesses not being addressed by FSMA.<sup>103</sup>

***Intentional contamination: is more resource allocation necessary?*** It has been three decades since a large-scale intentional adulteration of the food supply occurred.<sup>104</sup> While some “lone-wolf” actors have tried to contaminate soft targets,<sup>105</sup> and others abroad have used chemical contaminants to deceive regulatory requirements,<sup>106</sup> the intentional adulteration of a food product to achieve terroristic objectives simply has not occurred. Some scholars have stated that “in terms of pure statistical risk, the likelihood of [a biological weapon] causing a major event is very low simply because it is so difficult and comparatively very expensive for small-scale terrorist elements to manufacture, store, and deploy them.”<sup>107</sup> The policy recommendation is that DHS should continue to work with USDA and HHS to maintain a safe food supply, but increasing resource allocation toward an intentional adulteration of a food product is not warranted. The *status quo* should be maintained. The conventional systems currently in place to

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<sup>101</sup> Doyle et al.

<sup>102</sup> Ibid., p. 254.

<sup>103</sup> Ibid.

<sup>104</sup> Török et al.

<sup>105</sup> Tresa Baldas, "FBI Nabs Man Who Sprayed Mice Poison on Grocery Food in Ann Arbor," *Detroit Free Press* 2016.

<sup>106</sup> Barboza and Barrionuevo.

<sup>107</sup> Jane A. Bullock, George D. Haddow, and Damon P. Coppola, "Hazards," in *Introduction to Homeland Security, 5th Ed.* (Waltham, MA: Butterworth-Heineman, 2016)., p. 92.

control for unintentional contamination are sufficient to control an intentional contamination. As Ed You of the FBI Weapons of Mass Destruction directorate once said, “The most dangerous bioterrorist out there is Mother Nature.”<sup>108</sup>

#### **5.2.2.8. Tell Your Story.**

By going step-by-step through this process, the story is considered “told”, and Bardach’s process has been explore via our two contexts: FSMA and intentional contamination.

#### **5.2.3. Ethics and liability factors that influence policy development.**

The importance of, and reliance upon, ethics in food law is clearly seen in the very nature in which persons interact with the food system. All persons interact with the food system every day, often multiple times, and expectations are high.<sup>109</sup> Consumers expect food that is properly labeled, safe to consume, and exactly what the label purports it to be. As Sanchez (2015) states, the consumer-food interaction can best be summarized as “In Food We Trust”; modern society relies on others to produce their food and enforcement agencies (and the food producers themselves) to ensure products are not adulterated or misbranded.<sup>110</sup> Indeed, food is “an essential ingredient” of life, and food ethics uniquely “unites scholars with farmers and fishermen, chefs with industry, consumers with lawyers, and food citizens with authorities.”<sup>111</sup> Externalities, or the “ripple effect” of food-related issues, can cross industries and commodities.<sup>112</sup> For example, the Jensen Farms cantaloupe outbreak of 2011 impacted the profitability of other melon industries, including watermelon and honeydew.

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<sup>108</sup> Rowan Jacobsen, "The Ultimate Life Hack," *Outside*, September 2017., p. 74.

<sup>109</sup> Mark C. Sanchez, "Introduction to Statutory Framework and Case Law," in *Food Law and Regulation for Non-Lawyers: A U.S. Perspective*, ed. Mark C. Sanchez (Switzerland: Springer International Publishing, 2015).

<sup>110</sup> *Ibid.*

<sup>111</sup> Matthias Kaiser and Anne Algers, "Food Ethics: A Wide Field in Need of Dialogue," *Food Ethics* 1, no. 1 (2016)., p. 1.

<sup>112</sup> Sanchez.

The food system has a unique history, beginning with the manipulation of raw product for preservation or digestibility, to the rise in trade that tied civilizations together, to the industrialization of the food system that eliminated hunger and malnutrition.<sup>113</sup> Questions relating to food ethics must transverse a highly complex, global system. For example, rich, industrialized nations interact with poor, single-family producers in developing nations to import certain products. Or, decisions must be made related to food's impact on population growth, the interaction of food and climate change, the depletion of natural resources to grow and produce land- and sea-based foods, or the ramifications of trade.<sup>114</sup>

Food ethics also intimately interacts with animal sciences: issues relating to animal welfare, intensification of animal production practices, and the rise of antimicrobial resistance are topics in which the citizenry and food system actors alike find great interest.<sup>115</sup> It is also closely tied with issues of food safety, particularly those related to microbial contamination. Here, an interesting interaction exists between laws, market incentives, social norms, public health, and microbiological methods and detection.<sup>116</sup> What is *acceptable* “emerge[s] out of interactions among individuals and institutions throughout the network, and they evolve through feedback and learning.”<sup>117</sup> Control and regulation of bacterial contamination of food involves government, industry, and tort liability.<sup>118</sup>

In fact, the concept of liability becomes an important part of food ethics and safety. Liability, in this sense, can be defined as “the responsibility to pay compensation for damages

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<sup>113</sup> Kaiser and Algers.

<sup>114</sup> Ibid.

<sup>115</sup> Ibid.

<sup>116</sup> Timothy D. Lytton, "The Taming of the Stew: Regulatory Intermediaries in Food Safety Governance," *The Annals of the American Academy of Political and Social Science* 670, no. 1 (2017).

<sup>117</sup> Ibid., p. 79.

<sup>118</sup> Ibid.

such as caused by foodborne illness.”<sup>119</sup> Liability is best established through *traceability systems* and the ability to *take cases to court*.<sup>120,121</sup> From a traceability perspective, this means the ability to trace a product back to the farm or facility of origin and understand its path through the supply chain to the consumer.<sup>122</sup> Traceability permits companies to shift responsibility along the supply chain if necessary while giving consumers the ability to seek compensation for damages.<sup>123</sup>

In many ways, liability-related actions, pressures, and threats lead to an increase in food safety. Food companies often purchase liability insurance in the event of lawsuits stemming from a food safety crisis, and in turn insurers will incentivize companies to implement systems that reduce the likelihood of a food safety lapse. This can include underwriting food safety programs and lowering premium rates for companies with a strong food safety culture and history. Risk assessments, food safety programs, and third-party audits are thus conducted not only to prevent contamination, but to reduce liability and procure favorable insurance coverage.<sup>124</sup>

Where is the line drawn for ethical and liability responsibilities for the food industry, for regulators, or for private citizens? For example, the consumption of raw milk is a contentious issue in the United States: numerous states permit the sale of raw milk by various means (although it is illegal to sell across state lines), such as on-farm sales, in-store sales, or cow-sharing programs. Yet, epidemiological and microbiological evidence has confirmed time and again that unpasteurized milk is a health risk, and the number of outbreaks associated with raw milk consumption trumps those of pasteurized origin.<sup>125</sup> What is the ethical responsibility of a

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<sup>119</sup> Sébastien Pouliot and Daniel A. Sumner, "Traceability, Liability, and Incentives for Food Safety and Quality," *American Journal of Agricultural Economics* 90, no. 1 (2008).

<sup>120</sup> Ibid.

<sup>121</sup> Lytton.

<sup>122</sup> Pouliot and Sumner.

<sup>123</sup> Ibid.

<sup>124</sup> Lytton.

<sup>125</sup> Stephen P. Oliver et al., "Food Safety Hazards Associated with Consumption of Raw Milk," *Foodborne Pathogens and Disease* 6, no. 7 (2009). In fact, according to the authors, "the FDA, CDC, American Medical

farm selling raw milk? Who is liable if a child loses kidney function following a STEC infection from raw milk purchased by a parent? What about freedom-of-choice? These questions enter a debatable grey area in food ethics. Furthermore, will techniques such as uniform regulations and sanitation (as has worked in Europe<sup>126</sup>), labeling, and education be sufficient to legalize raw milk nationwide?<sup>127</sup>

Similar situations about consumer choice and food ethics and liability are seen throughout the system. The consumption of raw, ready-to-eat seafood is common (particularly sashimi, sushi, and raw oysters, all of which may pose a risk for *Bacillus cereus*, *Salmonella* spp., *E. coli*, *S. aureus*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*).<sup>128</sup> Raw sprouts, deemed a high-risk food by FDA, continue to appear on deli menus despite repeated outbreaks.<sup>129,130</sup> USDA encourages consumers to cook their ground beef to 160°F; nevertheless, many consumers continue to order burgers cooked medium or less, and wait staff in restaurants commonly recommend beef cooked to unsafe temperatures.<sup>131</sup> Regardless of the knowledge amassed regarding food safety risks, liability (and resultant litigation) is likely to continue as people become sick and courts continue to rule in favor of the sickened.<sup>132</sup>

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Association, American Academy of Pediatrics, American Public Health Association, U.S. Animal Health Association, USDA, National Environmental Health Association, International Association for Food Protection, and WHO, all have formal statements regarding the hazards of consumption of raw unpasteurized milk and advocate that milk be pasteurized" (p. 803).

<sup>126</sup> T. Yilmaz et al., "Outbreaks Associated with Unpasteurized Milk and Soft Cheese: An Overview of Consumer Safety," *Food Protection Trends* 29, no. 4 (2009).

<sup>127</sup> Oliver et al.

<sup>128</sup> H. W. Kim et al., "Raw Ready-to-Eat Seafood Safety: Microbiological Quality of the Various Seafood Species Available in Fishery, Hyper and Online Markets," *Letters in Applied Microbiology* 64, no. 1 (2017).

<sup>129</sup> R. M. Callejon et al., "Reported Foodborne Outbreaks Due to Fresh Produce in the United States and European Union: Trends and Causes," *Foodborne Pathogens and Disease* 12, no. 1 (2015).

<sup>130</sup> Food and Drug Administration, "FSMA Final Rule on Produce Safety".

<sup>131</sup> Ellen Marie Thomas, "Assessment of Risk Communication Associated with Ordering Undercooked Hamburgers in Restaurants" (North Carolina State University, 2015). On a personal note, the author enjoyed being on the same grant project (and the free lunches).

<sup>132</sup> William Marler, "Meet William Marler," <https://billmarler.com/biography>.

### 5.3. BEYOND FOOD SAFETY POLICY: FOOD DEFENSE, FOOD AND AGRICULTURE SECURITY, AND CRITICAL INFRASTRUCTURE PROTECTION

#### 5.3.1. Food defense at the facility level.

Food defense involves “the protection activities, and/or the security assurance process or procedures that deliver product safety with regard to intentional acts of adulteration.”<sup>133</sup> FDA expands this definition to “the effort to protect food from intentional acts of adulteration where *there is an intent to cause wide scale public harm.*”<sup>134</sup> Several systems for countering intentional contamination threats have been developed, including threat assessment critical control points (TACCP), vulnerability assessment critical control points (VACCP), the food protection risk matrix,<sup>135</sup> the NSF fraud protection model,<sup>136</sup> the USP preventative food fraud management system, and the CARVER + Shock tool.<sup>137,138</sup>

Food defense helps maintain public health and protects a company’s brand. Most importantly, strong food defense mechanisms reduce the likelihood of a bioterrorist attack on the food system. Such an attack would cause social panic, undermine confidence in the government, and negatively shock the economy.<sup>139,140</sup> Furthermore, “an attack via the food chain has a low

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<sup>133</sup> Louise Manning and Jan Mei Soon, "Food Safety, Food Fraud, and Food Defense: A Fast Evolving Literature," *Journal of Food Science* 81, no. 4 (2016).

<sup>134</sup> Department of Health and Human Services, "21 CFR Parts 11 and 121: Mitigation Strategies to Protect Food against Intentional Adulteration; Final Rule," ed. Department of Health and Human Services (2016). Emphasis added by the author.

<sup>135</sup> John Spink and Douglas C. Moyer, "Defining the Public Health Threat of Food Fraud," *Journal of Food Science* 76, no. 9 (2011).

<sup>136</sup> NSF Safety and Quality UK, "Risk Modeling of Food Fraud Motivation - Fs 246004 " <https://www.food.gov.uk/sites/default/files/NSF%20Final%20report.pdf>.

<sup>137</sup> Food and Drug Administration, "Carver + Shock Primer," (Washington, D.C.: Department of Health and Human Services, 2009).

<sup>138</sup> Manning and Soon.

<sup>139</sup> Chalk.

<sup>140</sup> Maynard.

barrier to entry and little skill needed to execute.”<sup>141</sup> While discussed prior to 9/11, food biosecurity evolved into a more prominent threat after the attacks.<sup>142</sup> In many ways, the threat still exists and the food system remains vulnerable. According to DHS and the Federal Bureau of Investigation (FBI), violent extremists and terrorists consider America’s agriculture and food production tempting targets and have indicated an interest in poisoning the food supply, which has great potential to cause costly economic losses and the supply chain for implicated foodstuffs, create public panic, and lead to a public health crisis with considerable mortality and morbidity.”<sup>143</sup><sup>144</sup> Fortunately, bipartisan action in Washington, D.C. has underscored that food defense is agnostic to politics, and DHS has channeled funding to anticipate and counter food safety threats.<sup>145,146,147</sup> Furthermore, FDA, in the multiple requirements to achieve the mandates dictated by FSMA, has promulgated the “Mitigation Strategies to Protect Food Against Intentional Adulteration” (hereafter referred to as “Mitigation Strategies Rule”).<sup>148</sup>

The final Mitigation Strategies Rule went into effect on May 27, 2016.<sup>149,150</sup> To comply with the Mitigation Strategies Rule, companies must develop a Food Defense Plan, which is comprised of the components described in Figure 5.1.

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<sup>141</sup> Greg Keeley and Kyle Landry, "Agroterrorism a Real Threat to America's Food Supply," *The Hill* 2017.

<sup>142</sup> Kennedy and Busta.

<sup>143</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan," (Washington, D.C.2015)., p. 5.

<sup>144</sup> Dean Olson and Federal Bureau of Investigation, "Agroterrorism: Threats to America's Economy and Food Supply," <https://leb.fbi.gov/articles/featured-articles/agroterrorism-threats-to-americas-economy-and-food-supply>.

<sup>145</sup> Obama.

<sup>146</sup> Food Safety News Desk.

<sup>147</sup> Department of Homeland Security.

<sup>148</sup> The Preventative Controls Rule also includes consideration of intentional adulteration risks.

<sup>149</sup> Department of Health and Human Services.

<sup>150</sup> "Key Requirements Fact Sheet: FSMA Final Rule for Mitigation Strategies to Protect Food against Intentional Adulteration," <https://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM503566.pdf>.

- 1) The written ***vulnerability assessment***, including required explanations, to identify significant vulnerabilities and actionable processes.
- 2) The written ***mitigation strategies***, including required explanations.
- 3) The written food defense ***monitoring procedures*** for the implementation of the mitigation strategies.
- 4) The written food defense ***corrective actions***.
- 5) The written food defense ***verification procedures***.

**Figure 5.1: Contents of a Food Defense Plan, as codified at 21 CFR § 121.126 (b)<sup>151</sup>**

The contents of a Food Defense Plan are similar to those contained within a HACCP plan. However, HACCP is designed to control *unintentional* contamination problems, and the systems in place could be *overridden* or *bypassed* in an intentional contamination event; thus, a somewhat modified system is necessary.<sup>152</sup> First, vulnerability assessments must occur at each step in the facility's process to evaluate (1) "the severity and scale of the potential impact on public health" (*e.g.*, volume of product, dissemination of product through supply chains, number of exposures, potential agents of concern), (2) general accessibility of the product and barriers in place, and (3) "the ability to successfully contaminate the product."<sup>153</sup> The importance of HACCP, GMPs, and "see something say something" practices should be underscored as well. Vulnerability assessments should be performed by qualified individuals.

A popular method to determine the current status of a facility's food defense capabilities is a CARVER+Shock analysis.<sup>154</sup> This tool "can be used to assess the vulnerabilities within a system or infrastructure to an attack."<sup>155</sup> Vulnerability is assessed for a food company as a whole, the vulnerability of specific buildings and/or entities within a company, or the

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<sup>151</sup> "21 CFR Parts 11 and 121: Mitigation Strategies to Protect Food against Intentional Adulteration; Final Rule."

<sup>152</sup> Kennedy and Busta.

<sup>153</sup> Department of Health and Human Services, "Key Requirements Fact Sheet: FSMA Final Rule for Mitigation Strategies to Protect Food against Intentional Adulteration".

<sup>154</sup> CARVER+Shock is not required by the Mitigation Strategies Rule. A facility can simply use the FDA-defined process steps instead.

<sup>155</sup> Food and Drug Administration, "Carver + Shock Primer."

vulnerability of a company's supply chain. Company leadership can better anticipate how "attractive" the company is to terrorists or subversive entities. A series of steps are performed in an effective CARVER + Shock analysis (Figure 5.2). For each node in the production system, a score is assigned for each C, A, R, V, E, R, and Shock parameter (scaled 1-10). Then, decisions are made by adding up each value of C, A, R, V, E, R, and Shock for each node, thus giving managerial staff starting point to reduce vulnerability.

| <b><u>COMPONENTS</u></b>   |                                                                                                            |
|----------------------------|------------------------------------------------------------------------------------------------------------|
| <b>C:</b>                  | Criticality – a measure of public health and economic impacts of an attack                                 |
| <b>A:</b>                  | Accessibility – ability to physically access and egress from target                                        |
| <b>R:</b>                  | Recuperability – ability of a system to recover from an attack                                             |
| <b>V:</b>                  | Vulnerability – ease of accomplishing attack                                                               |
| <b>E:</b>                  | Effect – amount of direct loss from an attack as measured by loss in production                            |
| <b>R:</b>                  | Recognizability – ease of identifying target                                                               |
| <b>Shock:</b>              | the combined health, economic, and psychological impacts of an attack                                      |
| <b><u>REQUIREMENTS</u></b> |                                                                                                            |
| 1.                         | <i>Establishing parameters</i> (What are you protecting? What are you protecting against? etc.)            |
| 2.                         | <i>Assembling experts</i> (Subject matter experts from food science, epidemiology, microbiology, etc.)     |
| 3.                         | <i>Detailing the food supply chain</i> (A flow chart of the system and its components/nodes)               |
| 4.                         | <i>Assigning scores</i> (Once broken down into its smaller parts, rank based on CARVER + Shock principles) |
| 5.                         | <i>Applying what has been learned</i> (Make changes in the critical nodes)                                 |

**Figure 5.2: Components for and requirements of a CARVER + Shock Analysis<sup>156</sup>**

The Mitigation Strategies Rule is an effective tool for food defense. Bolstering the entirety of food defense, however, also requires interactions with foreign actors and the U.S. import security system. Immediately following 9/11, Congress passed the *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* (the Bioterrorism Act). The Act

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<sup>156</sup> Ibid.

compels companies to ensure that foreign suppliers are providing prior notice of food shipments to FDA, that records are kept regarding foreign suppliers (and that the suppliers themselves are keeping records), and that supplies are being procured from registered facilities.<sup>157,158</sup> The policies and procedures of the Bioterrorism Act are intended to manage the health risks associated with internationally traded and produced agricultural products. FDA can detain products suspected to threaten human or animal health.<sup>159</sup> FSMA's final rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals makes U.S. owners or consignees of food offered for import to the U.S. responsible for "determining known or reasonably foreseeable hazards with each food; evaluating the risk posed by a food, based on the hazard analysis, and the foreign supplier's performance; using that evaluation of the risk posed by an imported food and the supplier's performance to approve suppliers and determine appropriate supplier verification activities; conducting supplier verification activities, and conducting corrective actions."<sup>160</sup>

### **5.3.2. The Department of Homeland Security and food defense.**

The United States prepares for a national food safety threat (and most biohazards in general) through a conglomeration of agencies, directorates, and personnel. DHS "leverages resources within federal, state, and local governments" and more than 87,000 different governmental jurisdictions to carry out DHS objectives.<sup>161</sup> DHS is mandated to implement policy

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<sup>157</sup> 107th Congress of the United States, "Public Health Security and Bioterrorism Preparedness and Response Act," in *Public Law 107-188*, ed. 107th Congress (2002).

<sup>158</sup> Shweta Gopalakrishnan et al., "The Multiplicity of Actors Involved in Securing America's Food Imports," *Journal of Homeland Security and Emergency Management* 9, no. 1 (2012).

<sup>159</sup> *Ibid.*

<sup>160</sup> Food and Drug Administration, "FSMA Final Rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals," <https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm361902.htm>.

<sup>161</sup> Jane A. Bullock, George D. Haddow, and Damon P. Coppola, "Governmental Homeland Security Structures," in *Introduction to Homeland Security, 5th Ed.* (Waltham, MA: Butterworth-Heinemann, 2016), p. 113.

and achieve objectives prescribed in Homeland Security Presidential Directives (HSPDs). Those that most impact food safety are given in Table 5-5. HHS coordinates functions for Public Health Emergency Preparedness and Disaster Response (HSPD-21). HHS (principally, FDA) and USDA are the sector-specific agencies for the Food and Agriculture sector (FA), partnering to address food safety and defense issues.<sup>162,163</sup>

**Table 5-5: Homeland Security Presidential Directives pertaining to food safety<sup>164</sup>**

| <b>HSPD</b>                                                                    | <b>Mission</b>                                                                                                                                                                                                                                                               |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HSPD-7: Critical Infrastructure Identification, Prioritization, and Protection | Establishes a national policy for federal departments and agencies to identify and prioritize United States critical infrastructure and key resources and to protect them from terrorist attacks.                                                                            |
| Presidential Policy Directive/PPD-8: National Preparedness                     | Aimed at strengthening the security and resilience of the United States through systematic preparation for the threats that pose the greatest risk to the security of the nation, including acts of terrorism, cyber-attacks, pandemics, and catastrophic natural disasters. |
| HSPD-9: Defense of United States Agriculture and Food                          | Establishes a national policy to defend the agriculture and food system against terrorist attacks, major disasters, and other emergencies.                                                                                                                                   |
| HSPD-10: Biodefense for the Twenty-First Century                               | Provides a comprehensive framework for the nation's biodefense                                                                                                                                                                                                               |

DHS is responsible for addressing terrorism as well as “a much wider portfolio of natural, technological, and intentional hazards” known by the acronym CBRNE: chemical, biological, radiological/nuclear, and explosive.<sup>165</sup> These CBRNE hazards, particularly chemical and biological, serve as particular threats to the food system. To address these crises emanating from these hazards, several agencies, offices, and directorates have been established. The Science and Technology (S&T) Directorate houses the Homeland Security Advanced Research Projects

<sup>162</sup> "Homeland Security: The Concept, the Organization," in *Introduction to Homeland Security, 5th Ed.* (Waltham, MA: Butterworth-Heinemann, 2016).

<sup>163</sup> Obama.

<sup>164</sup> Bullock, Haddow, and Coppola, "Homeland Security: The Concept, the Organization."

<sup>165</sup> "Hazards."

Agency (HSARPA), which manages the Chemical/Biological Defense Division.<sup>166,167</sup> The Chem/Bio Defense Division increases preparedness for chemical or biological threats through awareness, surveillance, and countermeasures. The Office of Health Affairs (OHA) coordinates the preparation for and response to medical-related emergencies, leading DHS's biodefense and food, agriculture, and veterinary defense activities (including the BioWatch early detection program).<sup>168,169</sup>

As aforementioned, a food safety challenge occurs at the nation's borders and seaports: the U.S. economy relies on the flow of goods in and out of the country, but the use of shipping containers as smuggling vehicles for dirty bombs or the importation of contaminated foods (or pests) poses a real threat. DHS implemented the Secure Freight Initiative (SFI) and Container Security Initiative (CSI) to address these threats.<sup>170</sup>

### **5.3.3. Food defense coordination with USDA, FDA, and other agencies.**

While DHS is seen as the primary anti-terrorism, homeland defense, and security-ensuring agency, it often coordinates with agencies that may have more expertise and functional capabilities to address homeland security initiatives and issues. As will be seen, USDA and FDA serve as agencies that work vis-à-vis DHS to achieve national security initiatives related to the food system.

USDA created the Office of Homeland Security and Emergency Coordination (OHSEC) in 2011 with six divisions to protect the nation's food supply and the infrastructures that assist

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<sup>166</sup> "Governmental Homeland Security Structures."

<sup>167</sup> Department of Homeland Security, "Science and Technology: Strategic Directions," <https://www.dhs.gov/science-and-technology/strategic-directions#>.

<sup>168</sup> Bullock, Haddow, and Coppola, "Governmental Homeland Security Structures."

<sup>169</sup> Department of Homeland Security, "The Biowatch Program," <https://www.dhs.gov/biowatch-program>.

<sup>170</sup> Bullock, Haddow, and Coppola, "Border Security, Immigration, and Customs Enforcement."

it.<sup>171</sup> The National Surveillance Unit within the Animal and Plant Health Inspection Service (APHIS) protects and monitors the health of livestock and poultry. FSIS “works to prevent, prepare for, respond to, and recover from non-routine emergencies resulting from intentional and unintentional contamination” in meat, poultry, and egg products.<sup>172</sup> FSIS also assesses food supply vulnerabilities, monitors the food supply for intentional contamination, and works with other government agencies to dictate proper responses. The Food Defense and Emergency Response division “serves as the lead coordinating body in the development of the infrastructure and capacity to prevent, prepare for, and respond to terrorism aimed at the U.S. food supply.”<sup>173,174</sup> USDA sponsors and evaluates several homeland security-applicable research projects, including research on rapid identification tests for biological agents; prevention, detection, and response efforts; laboratory safety; and the development of better detection for nontraditional weapons.<sup>175</sup>

Response to a food safety event starts with the National Response Framework (NRF), a “scalable, flexible, and adaptable” system that coordinates “the key roles and responsibilities of response participants throughout the country.”<sup>176</sup> Within the NRF are two food hazard-related incident annexes that apply the NRF: the Biological Incident Annex (coordinates the response to a disease outbreak of known or unknown origin, which could be disseminated through food) coordinated by HHS, and the Food and Agricultural Incident Annex (coordinates the response to emergencies involving the agricultural and food systems and determines the impact on food,

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<sup>171</sup> "Governmental Homeland Security Structures." The divisions are: Continuity and Planning Division (CPD), Emergency Programs Division (EPD), Personnel and Documents Safety Division (PDSD), Physical Security Division, Radiation Safety Division (RSD), Executive Protection Operations Division (EPO).

<sup>172</sup> *Ibid.*, p. 146.

<sup>173</sup> *Ibid.*, p. 146.

<sup>174</sup> United States Department of Agriculture, "Food Defense and Emergency Response " <https://www.fsis.usda.gov/wps/portal/fsis/topics/food-defense-defense-and-emergency-response>.

<sup>175</sup> Bullock, Haddow, and Coppola, "Governmental Homeland Security Structures."

<sup>176</sup> "All-Hazards Emergency Response and Recovery.", p. 442.

water, wildlife, and humans) coordinated by USDA and HHS.<sup>177,178,179</sup> Responses to a foodborne catastrophe would be further coordinated by the Federal Emergency Management Agency (FEMA; operational and logistical federal response), the Domestic Emergency Support Team (led by FBI; provides instruction on how to best respond to a biological hazard), the National Disaster Medical System (supplements localities with extra health personnel), and the FBI Laboratory Division (collection of hazardous or toxic evidence).<sup>180</sup> This literature review has focused on federal response, but inevitably state and local authorities would be involved as well. The author does not want to diminish their role and notes that DHS is involved in the coordination.

#### **5.3.4. Recognition and coordination.**

Wide-scale knowledge about biological threats and agents (and all CBRNE weapons) is minimal. Both the persons that would be responsible in crisis-response and the general public itself are under- or ill-informed about the CBRNE weapons and have limited capability in identifying and responding to their consequences.<sup>181</sup> Scientists and public health officials believe that best defense against a food safety threat is *recognition* that the hazard is actually occurring. To achieve this goal, physicians, first responders, epidemiologists, and others must be properly trained to have such recognition.<sup>182</sup> Recognition, however, is easier said than done. A food safety threat could involve a number of chemical, biological, or radiological hazard, with the biologic threat being primary. Biological weapons can be dispersed covertly into the food system, and the lag time between inoculation (dispersal) and appearance of symptoms can be

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<sup>177</sup> Ibid.

<sup>178</sup> Federal Emergency Management Agency, "Biological Incidence Annex," (Washington, D.C.: FEMA, 2008).

<sup>179</sup> "Food and Agricultural Incident Annex," (Washington, D.C.: FEMA, 2008).

<sup>180</sup> Bullock, Haddow, and Coppola, "All-Hazards Emergency Response and Recovery."

<sup>181</sup> Bullock, Haddow, and Coppola, "Hazards."

<sup>182</sup> Ibid.

days in duration. People will likely have no idea they have been exposed until the incubation time has elapsed.<sup>183</sup> One method to overcome the recognition hurdle is to vaccinate against certain threats. Unfortunately, such an avenue is not possible for high-risk pathogens and food threats: vaccines carry a high cost (*e.g.*, research, testing, and public relations).<sup>184</sup>

Controlling food safety threats is an important function of DHS and coordinating agencies. This coordination is a critical component for keeping Americans, and the food supply they use multiple times per day, safe. It is more likely that the next response coordinated by DHS, FDA, USDA, and/or other agencies will be to a “natural” event and not one perpetrated by a rogue terrorist cell. As the aforementioned Ed You of the FBI declared, “The most dangerous bioterrorist out there is Mother Nature.”<sup>185</sup> However, accounting for these threats and coordinating among agencies to control them, and those that are not yet known, is still critically important. The mental and physical tolls of an attack on the food system would reverberate throughout society. These interactions and coordination with other agencies leads to *greater resilience*, which is further discussed in chapter 6. They help shift unknown threats to known risks, a topic discussed in section 5.4.

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<sup>183</sup> Ibid. This discussion centers on human disease. A threat against the food supply could also involve targeting animals or plants. Human health may not be affected, but the economy certainly would.

<sup>184</sup> Ibid.

<sup>185</sup> Jacobsen., p. 74.

## 5.4. POLICY FRAMEWORKS TO ANTICIPATE “UNKNOWN UNKNOWN”

### 5.4.1. The Rumsfeld Dictum.

#### 5.4.1.1. Introduction.

At a Department of Defense (DOD) News Briefing in 2002, then Secretary of Defense Donald Rumsfeld made the following comment regarding the safety of America following the 9/11 attacks:

*“Reports that say that something hasn’t happened are always interesting to me, because as we know, there are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns -- the ones we don’t know we don’t know. And if one looks throughout the history of our country and other free countries, it is the latter category that tend to be the difficult ones.”<sup>186</sup>*

While Rumsfeld would use “unknown unknowns” as political ammunition in the lead up to and eventual invasion of Iraq, the concept he introduced (or re-introduced) that day is valuable across disciplines today. The concepts of “known unknowns” and “unknown unknowns” may serve as a valuable thinking framework for food regulators. What we know, what we think we know, and what we don’t know we don’t know can provide a modality to address food safety-related issues that were not on the public health “radar” 10, 15, or 25 years ago.

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<sup>186</sup> Donald Rumsfeld and Richard B. Myers, "Transcript: DOD News Briefing " <http://archive.defense.gov/Transcripts/Transcript.aspx?TranscriptID=2636>.. As evidenced by this citation, General Richard B. Myers was also part of the press briefing as part of his former role as Chairman of the Joint Chiefs of Staff. Myers, a distinguished alumnus of Kansas State University, is at the time of this writing serving as President of the University. Rumsfeld’s memoirs, published in 2011, are entitled *Known and Unknown: A Memoir*, and a documentary about him, directed by Errol Morris, is entitled “The Unknown Known”. In many ways, this statement became the credo of Rumsfeld’s legacy.

#### 5.4.1.2. Knowns and unknowns.

At Rumsfeld's initial "unknown unknowns" statement (hereafter referred to as Rumsfeld's Dictum), many in the press scoffed at his assertion;<sup>187</sup> yet, as time has passed, many in political science view Rumsfeld's words as "a profound distillation of the predicament of evidence-based policy."<sup>188</sup> Known knowns can be defined as the certain knowledge used to create programs and initiatives; known unknowns is the knowledge not fully known, but that which can be analyzed and used to make decisions based upon risk.<sup>189</sup> Finally, the "knowledge about what we do not know and cannot know" is the unknown unknown, a "wild card" of an event "that can throw over the most careful planning."<sup>190</sup>

Often, regulators expect evidence to present itself in "finite chunks offering certainty and security to policy decisions" (*a.k.a.* the known knowns), wherein empirical findings are assembled "to synthesize the available lessons on which initiatives and measures are the most fruitful."<sup>191</sup> Here, available knowledge is quantified into probabilistic and mathematical approaches to solve problems.<sup>192</sup> The problem with such an approach is that solely funneling empirical findings, database searches, and pre-established knowledge into policy decisions can result in a biased conclusions and paths forward.<sup>193</sup> A similar phenomenon happens in the hard sciences: after developing a hypothesis, researchers expect a range of known outcomes to occur following experimentation. On occasion, "the result is completely unexpected...: an unknown

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<sup>187</sup> David C. Logan, "Known Knowns, Known Unknowns, Unknown Unknowns and the Propagation of Scientific Enquiry," *Journal of Experimental Botany* 60, no. 3 (2009).; The term "universally lampooned" is used by this author.

<sup>188</sup> Ray Pawson, Geoff Wong, and Lesley Owen, "Known Knowns, Known Unknowns, Unknown Unknowns: The Predicament of Evidence-Based Policy," *American Journal of Evaluation* 32, no. 4 (2011)., p. 519.

<sup>189</sup> Christopher Daase and Oliver Kessler, "Knowns and Unknowns in the 'War on Terror': Uncertainty and the Political Construction of Danger," *Security Dialogue* 38, no. 4 (2007).

<sup>190</sup> *Ibid.*, p. 412.

<sup>191</sup> Pawson, Wong, and Owen., pp. 518-519.

<sup>192</sup> Daase and Kessler.

<sup>193</sup> Pawson, Wong, and Owen.

unknown.”<sup>194</sup> As an example, researchers investigating the phenomenon of intracellular protein targeting often use protein biochemistry techniques (an *in vitro* approach). However, when a group of researchers instead used a cell biological approach (an *in vivo* method), they uncovered a system of dual targeting by signal proteins to the endoplasmic reticulum and mitochondria that was impacted by a small tryptophan residue.<sup>195</sup> While the scientific ramifications for this are not relevant to this literature review, this example demonstrates that (1) unknown unknowns can impact both policy and scientific endeavors (so-called “hard” and “soft” sciences) and (2) the discovery of one unknown unknown reveals further questions to explore and advancements to make.<sup>196</sup>

In their analysis of knowns and unknowns, Daase and Kessler (2007) categorize the known known as a *threat*, the known unknown as a *risk*, and the unknown unknown as a *disaster*.<sup>197</sup> They add a fourth category as well: the unknown known, where facts are ignored or neglected, called *ignorance* (Figure 5.3).<sup>198</sup> As aforementioned, the known knowns are often analyzed by empirical knowledge and fact. The known unknown, or *risks*, are best analyzed through probabilistic knowledge, identifying not events but “trends and tendencies through the identification of conditions under which their realization is likely.”<sup>199</sup> Unknown unknowns result in instant, world-changing events that invalidate previous plans.<sup>200</sup>

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<sup>194</sup> Logan., p. 712.

<sup>195</sup> Ibid.; Laurent Chatre et al., "Efficient Mitochondrial Targeting Relies on Co-Operation of Multiple Protein Signals in Plants," *ibid.*

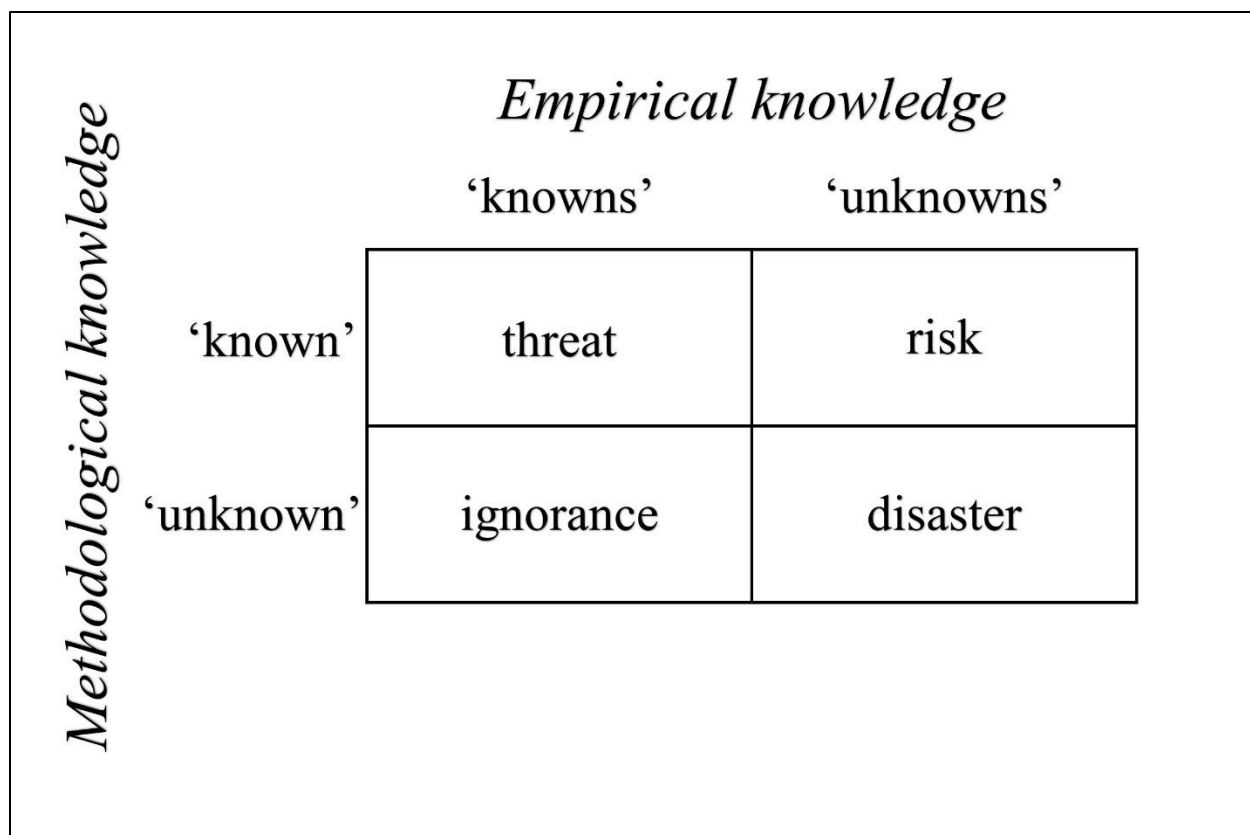
<sup>196</sup> David C. Logan, "Known Knowns, Known Unknowns, Unknown Unknowns and the Propagation of Scientific Enquiry," *ibid.*

<sup>197</sup> Daase and Kessler.

<sup>198</sup> Ibid.

<sup>199</sup> Ibid., p. 423.

<sup>200</sup> Ibid.



**Figure 5.3: Four kinds of danger.**

Author’s rendition of image created by Daase, Christopher, and Oliver Kessler. "Knowns and Unknowns in the 'War on Terror': Uncertainty and the Political Construction of Danger." *Security Dialogue* 38, no. 4 (2007): 411-34.

The goal, then, for policymakers (in any field) is to convert the unknowns to knowns.<sup>201</sup> In their paper on unknowns, Pawson et al. (2011) state that the “unknown unknowns” have been around for some time, as evidenced by a quote from former UK prime minister Harold MacMillian who, when asked what the “supreme challenge of government” was, replied “events, dear boy, events.”<sup>202</sup> Evidence and preparation can be minimized or rendered ineffectual in the instance of an event.<sup>203</sup>

<sup>201</sup> Pawson, Wong, and Owen.

<sup>202</sup> Ibid., p. 543.

<sup>203</sup> Daase and Kessler.

#### 5.4.1.3. Applications to food safety.

The concept of unknown unknowns can be applied to past events in the realm of food safety, both from an intentional and unintentional contamination perspective. While *Escherichia coli* was discovered in 1885, it was not identified as a foodborne pathogen until 1982.<sup>204</sup> Since that time, *E. coli* has transitioned from a “beef-only pathogen” to a pathogen found in a multitude of products.<sup>205</sup> The behavior of pathogenic *E. coli* is changing as well. For example, the *E. coli* O104:H4 outbreak in Germany was caused by an enteroaggregative *E. coli* that has acquired Shiga-toxin genes (as found in enterohemorrhagic *E. coli*), making an even more virulent pathogen.<sup>206</sup> This phenomenon is discussed further in chapter 6. In terms of intentional adulteration, the Rajneeshi contamination of salsa and salad bars,<sup>207</sup> the melamine scandal in pet food and infant formula in China,<sup>208</sup> and recent reports of rat poison being sprayed on salad bars at Whole Foods supermarkets in Michigan<sup>209</sup> serve as reminders of the vulnerability of the food system. Finally, as previously discussed in this dissertation, microbes in food are becoming resistant to antimicrobials, both antibiotics and sanitizers.<sup>210</sup>

#### 5.4.2. The World Trade Organization’s and the SPS Agreement’s acknowledgement of unknown unknowns.

The *Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)* began on January 1, 1995 in unison with the launching of the World Trade

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<sup>204</sup> Lee W. Riley et al., "Hemorrhagic Colitis Associated with a Rare *Escherichia coli* Serotype," *New England Journal of Medicine* 308, no. 12 (1983).

<sup>205</sup> For examples of implicated products, see Tables 5-1 and 5-2.

<sup>206</sup> Alexander Mellmann et al., "Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology," *PLoS ONE* 6, no. 7 (2011).

<sup>207</sup> Török et al.

<sup>208</sup> Bodeen.

<sup>209</sup> Baldas.

<sup>210</sup> Yuping Zhang et al., "Intracellular and Extracellular Antimicrobial Resistance Genes in the Sludge of Livestock Waste Management Structures," *Environmental Science & Technology* 47, no. 18 (2013).

Organization (WTO).<sup>211</sup> The *SPS Agreement* focuses on matters of food safety, animal health, and plant health in relation to international trade: nations are able to set their own safety standards as long as those standards are based on sound scientific principles and are not arbitrary, discriminatory, or scientifically unjustifiable.<sup>212,213</sup> Food safety issues are monitored under the standards and guidelines of the Codex Alimentarius Commission (Codex).<sup>214</sup> The international standards and guidelines for animal health are developed by the World Organization for Animal Health (the International Office of Epizootics; OIE), and the international standards for plant health are elaborated by the International Plant Protection Convention (IPPC).<sup>215</sup> Collectively, Codex, OIE, and IPPC form the “Three Sisters.”

The *SPS Agreement*, being an international agreement that must bridge differing cultures, infrastructures, and norms, must have a means to address the unknown unknowns. Article 5 concerns risk assessment and determination of the appropriate levels of sanitary or phytosanitary protection.<sup>216</sup> Article 5 encourages members to use scientific evidence, pursue relevant methods for inspection and testing, be fair regarding economic factors pertaining to protection, avoid arbitrary or unjustifiable protection measures, and prevent trade-restriction beyond a reasonable level.<sup>217</sup> Article 5.7 helps policymakers navigate “a nest of ambitions, a plethora of stakeholders, a sprawl of localities, and a checkering of histories” that influence food-related decision-making.<sup>218</sup> Specifically, Article 5.7 states:

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<sup>211</sup> World Trade Organization, “Understanding the WTO Agreement on Sanitary and Phytosanitary Measures ” [https://www.wto.org/english/tratop\\_e/sps\\_e/spsund\\_e.htm](https://www.wto.org/english/tratop_e/sps_e/spsund_e.htm).

<sup>212</sup> Ibid.

<sup>213</sup> “Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement),” (Geneva, Switzerland 1994).

<sup>214</sup> Ibid., Article 3.4

<sup>215</sup> Ibid.

<sup>216</sup> Ibid.

<sup>217</sup> Ibid.

<sup>218</sup> Pawson, Wong, and Owen., p. 541.

*In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time.*<sup>219</sup>

The invocation of Article 5.7 by a member institution permits a level of protection or safety in the case of an impending or ongoing “unknown unknown” or even an “unknown known” event. For example, following the Fukushima nuclear disaster, countries were concerned about importing Japanese fish, particularly when risk assessment data about the safety of the waters and fish was not readily available.<sup>220</sup> Article 5.7 provides a way for governments to institute caution for protective purposes.

#### **5.4.3. The rise of predictive modeling in food safety policymaking.**

Predictive modeling has become an important part of food safety policymaking. The ability to forecast food safety issues can help regulators, public health officials, private industry, and the general public. It can be argued that predictive modeling, and other cutting-edge tools, are essential for a food system that is more complex, interconnected, and global than ever before. Some argue that predicting survival, growth, and inactivation of pathogens in food is one of the greatest achievements in food safety the past twenty years.<sup>221</sup> Indeed, predictive modeling is one of the most effective tools to estimate “the changes in microbial levels in foods as [a] product moves through the farm-to-fork chain”, and such modeling is a bedrock component of risk

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<sup>219</sup> World Trade Organization, "Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)."

<sup>220</sup> Worland, Justin, “The Lingering Effects of Fukushima on Fish”. TIME Magazine. <http://time.com/4241443/fukushima-disaster-food-safety/>

<sup>221</sup> Konstantinos P. Koutsoumanis, Alexandra Lianou, and Maria Gougouli, "Latest Developments in Foodborne Pathogens Modeling," *Current Opinion in Food Science* 8 (2016).

assessment.<sup>222</sup> Current software used in microbial predictive modeling systems “offer a variety of utilities for the majority of foodborne pathogens including databases, fitting tools, predictions for growth, growth/no growth and inactivation, probabilistic models, and risk assessment modules” that can support HACCP, shelf-life studies, and experimental designs.<sup>223</sup> The models, however, are not perfect, and great variability exists in microbial ecosystems.<sup>224</sup> To account for this, Koutsoumanis et al. (2016) advocate stochastic modeling in which all possible conditions are monitored and risk levels are assigned.<sup>225</sup> Advancements in stochastic modeling have led to capabilities at the strain and even cellular levels.<sup>226</sup> Vilas et al. (2018) have designed a parameter identification protocol that helps define unknown parameters to better describe the physical, chemical, and biological changes in food processing.<sup>227</sup>

In a review for *Trends in Food Science & Technology*, Wang et al. (2016) discuss new modeling-based tools that food industry professionals can use to control food safety, including worldwide application of WGS, geographic information systems (GIS), and adaptable modeling tools.<sup>228</sup> Regarding WGS, the technology is becoming more commonplace in developing countries, allowing a better exchange of genetic information and more accurate characterization of pathogens.<sup>229</sup> GIS are computer systems that allow researchers “to manage, store, extract, organize, manipulate, and visualize data...for quantitative analysis and modeling.”<sup>230</sup> This allows

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<sup>222</sup> Ibid., p. 89.

<sup>223</sup> Ibid., p. 90.

<sup>224</sup> Codex Alimentarius Commission, "Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment," [www.fao.org/input/download/standards/10741/CXG\\_063e.pdf](http://www.fao.org/input/download/standards/10741/CXG_063e.pdf).

<sup>225</sup> Koutsoumanis, Lianou, and Gougouli.

<sup>226</sup> Ibid.

<sup>227</sup> Carlos Vilas et al., "Toward Predictive Food Process Models: A Protocol for Parameter Estimation," *Critical Reviews in Food Science and Nutrition* 58, no. 3 (2018).

<sup>228</sup> Siyun Wang et al., "Food Safety Trends: From Globalization of Whole Genome Sequencing to Application of New Tools to Prevent Foodborne Diseases," *Trends in Food Science & Technology* 57 (2016).

<sup>229</sup> Ibid.

<sup>230</sup> Ibid., pp. 190-191.

for a modern-day John Snow<sup>231</sup> to use spatial and non-spatial data, media, statistics, and other tools to better analyze large data sets.<sup>232</sup> The use of modeling tools adapted from landscape ecology, network analysis, and niche modeling has been advocated as well. Landscape ecology “recognizes that disturbances caused by anthropogenic or natural processes” may impact food safety, specifically the behavior of pathogens.<sup>233</sup> Social network analysis (SNA) can help in investigation outbreaks and product traceability.<sup>234</sup> Niche modeling examines climate changes that may be impacting food-related ecosystems, including the influence of global warming on the spread of foodborne disease and the effect of warming sea temperatures on seafood contamination with microorganisms such as *Vibrio* or algal bloom species.<sup>235,236</sup>

The use of “big data” to solve problems may have ramifications for the food industry as well; for example, supervised machine learning, where “software programs take as input training data sets and estimate or learn parameters that can be used to make predictions on new data,” including on where to deploy food safety inspectors, may revolutionize the industry.<sup>237</sup> Furthermore, the future may see a shift in modeling the growth/inactivation rates of bacteria (and other simple, observational data) to more complex realms, including physiological state and adaptive responses of bacteria.<sup>238</sup> This modeling will both give insight into microbial behavior

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<sup>231</sup> See: Steven Johnson, *The Ghost Map: The Story of London's Most Terrifying Epidemic--and How It Changed Science, Cities, and the Modern World* (New York City: Riverhead Books, 2007). As much as the author would like to take credit for the John Snow connection, credit is due to Wang et al.

<sup>232</sup> Wang et al.

<sup>233</sup> Ibid., p. 192.

<sup>234</sup> Ibid.

<sup>235</sup> Ibid.

<sup>236</sup> Antarpreet S. Jutla, Ali S. Akanda, and Shafiqul Islam, "A Framework for Predicting Endemic Cholera Using Satellite Derived Environmental Determinants," *Environmental Modelling & Software* 47 (2013).

<sup>237</sup> Susan Athey, "Beyond Prediction: Using Big Data for Policy Problems," *Science* 355, no. 6324 (2017).

<sup>238</sup> Koutsoumanis, Lianou, and Gougouli.

and shed light on key intracellular information.<sup>239,240,241</sup> Correlating current predictive models with realistic conditions will shift modeling from a “black-box” approach to one that understands the underlying mechanisms, or the “white box”.<sup>242</sup>

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<sup>239</sup> Ibid.

<sup>240</sup> TA McMeekin, LA Mellefont, and T Ross, "Predictive Microbiology: Past, Present and Future," *Modelling Microorganisms in Food* 1 (2007).

<sup>241</sup> J. F. Van Impe, D. Vercammen, and E. Van Derlinden, "Toward a Next Generation of Predictive Models: A Systems Biology Primer," *Food Control* 29, no. 2 (2013).

<sup>242</sup> Ibid.

## Chapter 6 - Policy Analysis with Resilience in Mind

### 6.1. ABSTRACT

In 2004, the Bush administration adopted Homeland Security Presidential Directive 9 (HSPD-9) establishing a national policy to protect American agriculture and the safety and security of the United States (U.S.) food supply. As part of this directive, the U.S. Food and Agriculture Sector (FA) was given “Critical Infrastructure and Key Resources” status; the directive effectively declared that American agriculture and the U.S. food supply are, for an array of reasons, essential to the American way of life. Thereafter, Presidential Policy Directive 21 (PPD 21) affirmed this status, subtly changing the name to a critical infrastructure. The U.S. Department of Homeland Security, the agency responsible for overseeing critical infrastructures, especially values *resilience* including FA. In addition to DHS, innumerable public agencies as well as private actors (including, but not limited to, food processing firms) view resilience as one prerequisite to maintaining the public’s *confidence* in the safety and security of the food supply. Several economic, social, and political factors drive this relationship between *resilience* and *consumer confidence*. If a major food catastrophe (*e.g.*, an intentional or unintentional adulteration of a food product causing large-scale illness, disability, and loss of life), a menu of public, private, and third-sector actors would be involved in ensuring resilience. Past U.S.-based food safety related events involving overseas offenders confirm this. Public and private actors with enforcement and risk-management responsibility will continue to be called upon to ensure resilience and, significantly, ensure consumer confidence. Most importantly, the response of private actors (*e.g.*, the involved or implicated manufacturer(s), the retailer(s), as well as consumers) would be most determinative of a successfully resilient food-system response. As

argued over the last decade by DHS, public-private partnerships remain critical to the FA critical infrastructure.

## 6.2. INTRODUCTION

If true security is achieved through “the reduction of terrorism and the ability to pursue and maintain social practices and opportunities that Americans hold dear,” then the raising, growing, production, distribution, and consumption of food must be protected.<sup>243</sup> American agriculture and the food system together form an essential pillar of the American way of life, and U.S. homeland security policy affirms this essentialism by the designation of food and agriculture (FA) as a *critical infrastructure sector* (akin to CIKR) under Presidential Policy Directive 21 (PPD 21).<sup>244,245</sup> Through public-private partnerships, multiple regulation- and policy-coordinating bodies, and an overall Department of Homeland Security (DHS) Sector-Specific Plan (SSP), the U.S. federal government seeks to ensure the security and resilience of FA.<sup>246</sup> Under PPD 21, USDA and HHS are the sector specific agencies for the FA sector. As per PPD 21, they have unique roles and responsibilities, vis-à-vis DHS, in ensuring critical infrastructure protection. Furthermore, in July 2017 Congress passed and the President signed an amendment to the 2002 *Homeland Security Act* that focuses on coordinating DHS efforts to protect food and agriculture through oversight and integration of programs and preparedness measures.<sup>247,248</sup> Scholars of homeland security ought to address whether this approach is

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<sup>243</sup> Annette D. Beresford, "Homeland Security as an American Ideology: Implications for U.S. Policy and Action," *Journal of Homeland Security and Emergency Management* 1, no. 3 (2004), p. 16.

<sup>244</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan."

<sup>245</sup> Obama.

<sup>246</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan."

<sup>247</sup> Food Safety News Desk.

<sup>248</sup> 115th Congress of the United States.

sufficient and, speculating on the future, what would happen if the safety, security, and operation of the FA was challenged?

This chapter explores these questions in terms of the *resilience* of FA, specifically assessing how FA would handle a food catastrophe. The demands of such an event on FA's resilience is contemplated based on historical examples and current systems. If FA—or any critical infrastructure sector—can increase its resiliency, it is more likely “to withstand adversity and to recover more quickly” if there was a major catastrophe.<sup>249</sup> Cutter et al. (2010) state that resilience involves “social, economic, institutional, infrastructural, ecological, and community elements.”<sup>250</sup> While all of these elements are important, this paper focusses on institutions and infrastructure, concluding with a commentary on which institutions and actors are in the best position to exercise leadership for ensuring food-system resilience.

### **6.3. CHALLENGES TO FOOD AND AGRICULTURE RESILIENCE**

Food catastrophes<sup>251</sup> can be intentional or unintentional.<sup>252</sup> While homeland security experts must focus on an intentional attack, both intentional *and/or* unintentional acts can test the sector's resiliency; historical examples of both kinds of acts offer insights for a resiliency assessment.

Intentional food catastrophes are usually terroristic or economic in nature.<sup>253,254</sup> By its very nature, the food system, with its scope, size, and importance to daily life, is an enticing

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<sup>249</sup> Susan L. Cutter, Christopher G. Burton, and Christopher T. Emrich, "Disaster Resilience Indicators for Benchmarking Baseline Conditions," *Journal of Homeland Security and Emergency Management* 7, no. 1 (2010)., p. 1.

<sup>250</sup> Ibid., p. 6.

<sup>251</sup> From this point forward, we refer to “catastrophe” as being a major food contamination event, likely from microbial or chemical sources.

<sup>252</sup> Kennedy and Busta.

<sup>253</sup> Jerry Jaax, "The Bio/Agro Terrorist Threat," in *A Multidisciplinary View of Food Safety and Security* (Manhattan, KS: Kansas State University, 2004).

<sup>254</sup> Food terrorism is “an act or threat of deliberate contamination of food for human consumption with chemical, biological or radionuclear agents for the purpose of causing injury or death to civilian populations and/or disrupting

target for a terrorist attack. Some have argued that FA is at risk because American agriculture is both widespread (*e.g.*, large fields with lax security) and concentrated (*e.g.*, cattle feeding operations contain many animals in a small area), susceptible to various threat agents (*e.g.*, microorganisms and toxins that are harmful to plants, animals, and/or humans), and incredibly important to several supply chains.<sup>255,256</sup> Yet, an intentional attack has yet to occur. Major outbreaks have been the result of lapses in hazard control programs or simply the result of living in a microbial world.<sup>257</sup> Thus, the risk exists, and given the landscape of risk (be it resulting from a bad actor or poor practices) mandates the need for resilience. It is precisely risk (*i.e.*, intentional contamination of the food supply for the express purpose of causing significant public health impact and harm) that the FSMA Final Rule for Mitigation Strategies to Protect Food Against Intentional Adulteration is intended to address. Given the pioneering nature of the provisions of this rule, the development and implementation of food defense planning efforts has and will continue to be an evolving focus for the food industry and regulators alike.

Intentional adulteration of food by a terrorist is not the only threat facing the United States. Economically motivated adulteration (EMA) occurs when inferior ingredients are used to deceive regulators or the public by using a similar, cheaper alternative.<sup>258</sup> A relatively recent

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social, economic or political stability.” (see: World Health Organization, “Terrorist Threats to Food: Guidance for Establishing and Strengthening Prevention and Response Systems,” in *Food Safety Issues* (Geneva, Switzerland 2008).)

<sup>255</sup> Chalk.

<sup>256</sup> Maynard.

<sup>257</sup> Kennedy and Busta. Note: Hazard control typically occurs under the guidance of a HACCP program, which stands for Hazard Analysis Critical Control Points, a preventative approach to eliminate chemical, biological, and physical hazards in food production.

<sup>258</sup> Sanchez, “Adulteration.” In fact, economic adulteration came to be following the allegations made in Upton Sinclair’s *The Jungle* (1904-1906).

example of EMA is the use of melamine in pet food and infant formula in China.<sup>259,260,261</sup> The melamine scandal highlighted both the impact of poor resilience in the absence of traceability and the ramifications of a highly segmented supply chain. This paper further explores such challenges related to traceability gaps and segmented supply chains.

The intentional adulteration—whether terrorism-related or economically-motivated—of food can have widespread impacts. Since such events are rare, and thus, examining unintentional adulteration is needed to empirically gauge FA resilience. One concern in an intentional contamination is the use of a “new” agent previously unseen, and the 2011 *Escherichia coli* O104:H4 outbreak in sprouts demonstrates such an event. This particular *E. coli* strain acquired new genes that made its initial microbiological classification difficult (i.e., it had never caused illness in animals prior, and the unseen genes muddled epidemiological investigations) and afforded the microbe the capability to cause severe sequelae.<sup>262</sup> The intentional introduction of a genetically modified or novel microbe could cause similar confusion and response delays.

Another area-of-concern regarding an intentional contamination event is the likelihood that other industries will be economically affected. Public health scientists have seen this through past cases of unintentional contamination. During a major outbreak of *Salmonella* Saintpaul in May 2008 in the United States, the CDC initially implicated red plum, Roma, and red round tomatoes. As time progressed, more cases were reported and further scientific analysis was conducted, prompting the CDC to include cilantro and fresh hot chili peppers (such as jalapeños) in the outbreak. By July 2008, tomatoes had been cleared as the contamination vehicle, but the

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<sup>259</sup> Melamine behaves similar to protein in certain analytical tests. Thus, by supplementing a product with melamine, a producer can increase the perceived protein content without actually increasing protein.

<sup>260</sup> Nearly 60 million packages of pet food exported to the U.S. were recalled after 14,000 pets were sickened (see: Barboza and Barrionuevo.)

<sup>261</sup> The use of melamine in infant formula resulted in the death of six infants and sickened 294,000. Alcorn and Ouyang.

<sup>262</sup> Mellmann et al. This severe sequelae included 830 cases of hemolytic uremic syndrome (HUS) and 46 deaths.

damage to several food brands had already been done—the tomato industry suffered an estimated \$250 million in losses as a result of the outbreak. “Finger-pointing” in a food safety outbreak can quickly implicate—and impact—an otherwise not-guilty commodity.<sup>263</sup> The 2011 *Listeria monocytogenes* outbreak in cantaloupe that damaged other melon industries is another example of the “finger-pointing” externality. While all *Listeria* cases were traced back to a cantaloupe farm in Colorado, the national watermelon, honeydew, and cantaloupe industries were all affected. The economic and social effects—workers laid off, brand images damaged, profit lost—must be considered when assessing FA resilience to a major food catastrophe.<sup>264</sup>

#### **6.4. THE CURRENT RESILIENCE OF THE FOOD AND AGRICULTURE SECTOR**

An FA catastrophe is indeed possible. During Operation Enduring Freedom, documents were recovered from an al-Qaeda hideout that discussed bioweaponization schemes and listed potential biowarfare agents.<sup>265</sup> In 2004, former Secretary of Health and Human Services Tommy Thompson remarked, “For the life of me, I cannot understand why the terrorists, have not ... attacked our food supply, because it is so easy to do.”<sup>266</sup> Furthermore, a RAND Company analysis regarding a biological attack on the food supply stated that “the United States, more by luck than by design, has not experienced the type of ... food-related disasters to which other countries ... have been subjected in recent years.”<sup>267</sup>

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<sup>263</sup> Kathryn Krusemark and Cobus Block, “Historical and Contemporary Cases Illustrating the Vulnerability of Specific Commodities and Sectors,” in *Food and Agricultural Security: An Historical, Multidisciplinary Approach*, ed. Justin Kastner (Santa Barbara, CA: ABC-CLIO, 2011).

<sup>264</sup> Sanchez, “Introduction to Statutory Framework and Case Law.”

<sup>265</sup> Jaax.

<sup>266</sup> FoxNews.com, “Attacking the U.S. Food Supply,” <http://www.foxnews.com/story/2004/12/09/attacking-us-food-supply/>.

<sup>267</sup> Chalk., p. xii. Those countries are the UK, Malaysia, and Taiwan.

FA is vulnerable to an attack for many reasons: the industry is comprised of “concentrated and intensive” practices that are then conveyed through a “highly dispersed” system with surveillance and response capabilities that are lacking at-best.<sup>268</sup> Modern food supply chains are complex, and sudden disruptions can have reverberations down the supply chain and potentially cause “widespread economic, political, and social disruptions.”<sup>269</sup> Furthermore, food companies are becoming more reliant on information-technology (IT) systems, and computers can be hacked (as seen in a recent, major computer glitch that impacted Starbucks).<sup>270</sup> Indeed, IT-based industrial control systems (routinely used for product formulation in food processing plants) feature potential cyber-vulnerabilities.

Oversight of the food system, particularly food safety oversight, is reasonably fragmented in the United States. A recent report from the Government Accountability Office (GAO) laments the fact that 16 federal agencies oversee 30 federal laws ultimately leads to “inconsistent oversight, ineffective coordination, and inefficient use of resources.”<sup>271</sup> This fragmentation may make the U.S. FA vulnerable to an intentional contamination event, or less resilient, or both.

An intentional contamination event would likely be chemical or microbiological in nature.<sup>272</sup> Introducing a pathogen into the supply chain requires compatibility between the microbe and the food product in which stability of both is maintained and organoleptic changes

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<sup>268</sup> Ibid.

<sup>269</sup> Maynard., p. 2.

<sup>270</sup> Anonymous, "Starbucks Computer System Crashes," CBS Chicago, <http://chicago.cbslocal.com/2017/05/16/starbucks-computer-system-crashes-computer-hack/>.

<sup>271</sup> United States Government Accountability Office, "Food Safety: A National Strategy Is Needed to Address Fragmentation in Federal Oversight," (Washington, D.C.: GAO-17-74, 2017).

<sup>272</sup> For microbial-based contaminations, the reasons for this are ease of growth and reproduction—a single colony can be replicated in a basic laboratory setting many times over (the use of *Salmonella* in The Dalles, Oregon, in 1984 shows such precedence). For chemical-based contaminations, the melamine scandal in China demonstrates that such an event is possible.

in the food do not occur.<sup>273</sup> Microorganisms (and associated toxins) listed as “Select Agents”<sup>274</sup> that are uncommonly associated with foods are of greatest concern.<sup>275</sup> An examination of food safety plans (known in the industry as HACCP plans) may illuminate vulnerabilities in the system. HACCP should control for unintended catastrophes. However, for intentional contamination, HACCP systems in place could be bypassed.<sup>276</sup> Conducting a hazard analysis is a critical step in HACCP plan development that aims to identify significant hazards, which are hazards that are both likely to occur and cause consumers harm that must be addressed in the HACCP plan in order to produce a safe food product.<sup>277</sup> HACCP may be used as a tool to address both intentional and unintentional contamination of food; however, including intentional contamination is not a requirement of the HACCP process. While intentional contamination may be deterred or detected by the numerous precautions that are taken to assure food safety, it is also important to specifically consider who the perpetrator might be in an intentional contamination event, what their motivation might be, as well as the impact of such an attack in order to more effectively use HACCP to address intentional contamination scenarios.<sup>278</sup>

While Select Agents could cause large-scale loss of life, the financial impact of a large outbreak, be it caused by a Select Agent or not, can be similarly enormous: “The fiscal downstream effect of a major act of sabotage against the food industry would [could] be multidimensional, reverberating through other sectors of the U.S. economy and ultimately

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<sup>273</sup> Kennedy and Busta.

<sup>274</sup> Select-agent status is determined by CDC and includes a wide variety of pathogens and associated toxins, including botulinum neurotoxins, hemorrhagic viruses, ricin, and pathogens that are highly damaging to plants and livestock. (see: 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73).

<sup>275</sup> Kennedy and Busta.

<sup>276</sup> Ibid. This “by-passing” could include the use of microorganisms not accounted for in the hazard analysis or having an insider disable certain hazard-controlling production processes.

<sup>277</sup> Carol A. Wallace, William H. Sperber, and Sara E. Mortimore, “Developing a HACCP Plan,” in *Food Safety for the 21st Century* (Chichester, West Sussex, United Kingdom: Wiley-Blackwell, 2011).

<sup>278</sup> Manning and Soon.

impacting directly on the American consumer.”<sup>279</sup> Indeed, terrorists are likely to target a food product with “lingering potential impact on consumer confidence and the economy.”<sup>280</sup>

In the event of an intentional food catastrophe, the first task for U.S. leadership would be to deploy public health resources and agencies, including leadership from DHS, HHS (particularly the Food and Drug Administration [FDA]),<sup>281</sup> USDA (particularly the Food Safety and Inspection Service, and the Animal and Plant Health Inspection Service), the CDC, and others.<sup>282</sup> Other key regulatory actors, such as state and local departments of health, would be involved, too. Assuming an event of international (and trade-related) scope, such multilateral bodies as the World Trade Organization (based in Geneva, Switzerland) and the food safety standard-setting Codex Alimentarius Commission (based in Rome, Italy) would have an indirect, but neither direct nor immediate, role to play; instead, the nation-state governments of involved trading partners would be most involved, and cross-border cooperation (e.g., between U.S. agencies and their counterparts in other countries) would be an important part of resilience. Following the realization of the catastrophe, a product recall would be both necessary and complex. The stress placed on U.S. food transport networks in such a scenario could hinder the speed of recalls, and the already-difficult task of locating specific products along complex global supply chains could be made harder by rising political instability elsewhere in the system. These “positive feedback” effects could serve to add complexity and cost to a product recall.”<sup>283</sup>

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<sup>279</sup> Chalk., p. 5.

<sup>280</sup> Kennedy and Busta., p. 92.

<sup>281</sup> In fact, the FDA has recently issued the Mitigation Strategies to Protect Food Against Intentional Adulteration Final Rule, which will be discussed in this paper’s presentation.

<sup>282</sup> DHS: Department of Homeland Security; HHS: Health and Human Services; USDA: United States Department of Agriculture; FSIS: Food Safety and Inspection Service; APHIS: Animal and Plant Health Inspection Service; CDC: Centers for Disease Control and Prevention.

<sup>283</sup> Maynard.

Next, assuaging public concern and demonstrating that the U.S. is “in control” would be important. In fact, resilience *must* be assured, because a major outbreak could damage the trust of the government and foment social instability. Attacks could “elicit fear and anxiety.”<sup>284</sup> An attack on livestock used for human food is concerning, too. Public angst would increase as “[g]raphic images of diseased cows and sheep would likely appear in the media, serving to demonstrate the extreme susceptibility of animals to disease and the vulnerability of all animal life, including humans, to deadly pathogens.”<sup>285</sup>

### **6.5. MOVING FORWARD: WHO IS IN THE BEST POSITION TO EXERCISE LEADERSHIP IN FOOD AND AGRICULTURAL RESILIENCE?**

Who, or what institution, is in the best position to lead in a time of crisis? The crisis, first and foremost, will be a time of tremendous public anxiety. *Resilience*, one might argue, would involve institutional leaders responding in the midst of such anxiety. The late scholar Edwin H. Friedman states that there are five aspects of anxiety in society, the most critical aspect being a lack of what he terms *well-differentiated leadership*.<sup>286</sup> Well-differentiated leadership is true (effective) leadership, and it involves what Friedman terms “self-regulation,” “adaptation to strength,” “response to challenge,” and the “capability to allow time for processes to occur.”<sup>287</sup> In the case of a food catastrophe of international origin, the stakeholders themselves (particularly private actors) are in the best position to exercise well-differentiated leadership. Food corporations, including transnational firms, connected with an intentional catastrophe must deploy resources to address the problem, and the work of public-private partnerships must

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<sup>284</sup> Chalk., p. 25.

<sup>285</sup> Ibid., p. 22.

<sup>286</sup> Edwin H. Friedman, *A Failure of Nerve: Leadership in the Age of the Quick Fix* (New York: Seabury Books, 2007). The other four are reactivity, herding, blaming, and a quick-fix mentality.

<sup>287</sup> Ibid., p. 24.

flourish. The interplay between DHS, USDA, and HHS is critical to improve and exercise resilience in light of an event.

Shocks to FA can last for many years.<sup>288</sup> To bolster resilience, regulators must first understand the risk. They must learn to model and quantify the impact of a catastrophe on the implicated food market and the subsequent ripples in related industries.<sup>289</sup> Of course, this raises the problem of forecasting the catastrophe—one cannot know which industry it will be nor the magnitude of a future event. The science (and maybe better put, the “art”) of seeking out *unknown unknowns* to better characterize risk should be developed (see section 5.4 *et seq.* of this dissertation).

Scholars must “explore opportunities for coordinated risk management” in which “governments, international institutions, and businesses develop contingency plans and establish early warning systems.”<sup>290</sup> Multilateral organizations such as Codex could contribute by developing methodologies to do this. Resilience includes “good PR”: the Chilean grape scare is an example of “how poor risk and crisis communication can cause far more damage to the economy than any actual terrorist incident.”<sup>291</sup>

Finally, the U.S. must “invest in strategic storage.”<sup>292</sup> This includes storage not only of medical stockpiles (for both humans and livestock) but also stockpiles of food and seeds (in the case of a major plant pathogen). Regardless of the food product implicated or the agent used,

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<sup>288</sup> Maynard.

<sup>289</sup> Rob Bailey et al., “Extreme Weather and Resilience of the Global Food System,” in *Final Project Report from the UK-US Taskforce on Extreme Weather and Global Food System Resilience*, ed. The Global Food Security Programme (UK2015).

<sup>290</sup> *Ibid.*, p.2.

<sup>291</sup> G.R. Dalziel, “Food Defence Incidents 1950-2008: A Chronology and Analysis of Incidents Involving the Malicious Contamination of the Food Supply Chain,” (Centre of Excellence for National Security (CENS): S. Rajaratnam School of International Studies at Nanyang Technological University 2009)., p. 12. Briefly, someone called the U.S. embassy in Santiago claiming grapes destined for export were poisoned with cyanide. Only trace amounts were found.

<sup>292</sup> Bailey et al., p. 11.

public-private partnerships must exercise strong leadership to foster resiliency. Supremely, while DHS must play a coordinating role, it will be private firms that are in the best position to exercise leadership. Nevertheless, such sub-agency units as the DHS Office of the Inspector General (OIG), must be involved. As documented in a 2007 report, the DHS OIG is federally mandated to audit and monitor the effectiveness and efficiency of DHS and its work; we posit that future auditing and monitoring by the OIG should explicitly involve an examination of public-private partnership effectiveness. While progress was made early on to both establish a Food Information Sharing and Analysis Center (ISAC) and engage with logical industry partners (e.g., the Food Marketing Institute),<sup>293</sup> further work needs to be done to ensure that all private stakeholders are indeed engaged in an overall food-system resilience strategy.

## 6.6. CONCLUSION

While policy efforts must continue in Washington, it will take public-private partnerships and innovation in food safety systems to ensure the safety of the American food supply from coast to coast and border to border. As one industry leader aptly stated, “[t]here can’t be national security without a secure food supply.”<sup>294</sup> Unfortunately, that national security faces challenges that are ever-present. While this chapter has explored both the threat of a food system catastrophe and the resiliency of the system, it is important to realize that, as authors Gary LaFree and Martha Crenshaw state, there is no simple solution to terrorism.<sup>295</sup> Namely, counterterrorism efforts “must succeed all the time, the terrorist only once.”<sup>296</sup> It is indeed

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<sup>293</sup> Department of Homeland Security Office of the Inspector General, *The Department of Homeland Security's Role in Food Defense and Critical Infrastructure Protection* (Washington, D.C.: U.S. Department of Homeland Security, 2007), p. 31.

<sup>294</sup> Curtis Meier, "Opinion: We Must Do More to Protect Our Farms from Terror Threats," *The Des Moines Register* 2017.

<sup>295</sup> Anonymous, "No Simple Solutions for Counterterrorism: LaFree and Crenshaw Book Talk Feb. 23," START <http://www.start.umd.edu/news/no-simple-solutions-counterterrorism>.

<sup>296</sup> Ibid.

possible for America to enhance its food-system resilience through the actions of well-differentiated leading institutions and robust public-private partnerships.

## Chapter 7 - Policy Development for Unknown Unknowns: Strategies and Lessons

### 7.1. INTRODUCTION

The previous chapter addressed the need for having a resilient food system. Indeed, before advocating for using prediction or forecasting, one must first ensure the overall resilience of the system. Because of its scale and complexity, the American food system will be resilient only to the extent that both effective regulatory cooperation (between and among the agencies involved) and vibrant public-private partnerships persist (see the conclusion of chapter 6). However, even if a resilient food system is assumed, policymakers must still grapple with the problem of unknown unknowns (introduced in 5.4 *et seq.*). Delving into the concept of forecasting and unveiling unknown unknowns is a worthwhile task. If the U.S. can become nimbler in thinking about and looking out for indicators of unknown unknowns, the U.S. food system can become even more resilient.

This chapter proposes five legitimate strategies for policymakers to use in the ever-important quest to identify the who, what, where, when, and why of the next food safety crisis. In doing so, policymakers can make efforts are made at solving “Rumsfeld’s Dictum,” or anticipating *what they do not know they do not know* (the unknown unknown, or disasters). In doing so, policymakers will likely shed light on issues regarding that which *they know they do not know* (the known unknown, or risk), helping to increase the overall safety, reliability, and future of the food system.<sup>297,298,299,300</sup> The strategies advocated in this chapter are given in Figure 7.1.

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<sup>297</sup> Rumsfeld and Myers.

<sup>298</sup> Daase and Kessler.

<sup>299</sup> Pawson, Wong, and Owen.

<sup>300</sup> Logan.

- 1) Learn from the past.
- 2) Target food groups of high and/or increasing consumption.
- 3) Assess FA threats primarily rooted in other critical infrastructures.
- 4) Borrow concepts and principles from meteorological forecasting.
- 5) Advocate multidisciplinary thinking.

**Figure 7.1: Strategies to identify and respond to Unknown Unknowns in the food safety realm**

As these strategies are discussed, three key terms should be kept in mind.<sup>301</sup> The first is ***serendipity***. Serendipity is the occurrence and development of events by chance in a happy or beneficial way. Advances in many academic fields have been serendipitous, and fostering an appreciation for learning will only increase serendipitous events. Throughout the history of science and the history of public health, solutions have been discovered and hazards averted through serendipity. The next word is ***speculate***. To speculate is to form a theory or conjecture without firm evidence. It can be argued that the exercise of thinking about unknown unknowns is just that—an exercise in speculating. Speculation is not a waste of time but rather a worthwhile endeavor. Speculating makes the mind limber and encourages one to *get outside the box*. The final term is the aforementioned ***resilience***, or the capacity to recover quickly from difficulties (toughness or elasticity). In many ways, being resilient helps policymakers both speculate on future crises and be open to serendipitous discoveries; in turn, by speculating and being open to serendipitous discoveries, we find more ways to be resilient. Americans, and global citizens in general, strive to be resilient people, to raise resilient children, and to live in a resilient country. This chapter will hopefully increase the “resilience quotient” of the food system we hold dear.

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<sup>301</sup> The definitions are provided by Google.

## 7.2. STRATEGY 1: LEARN FROM THE PAST

### 7.2.1. Introduction.

The 2011 *Escherichia coli* O104:H4 outbreak in Germany and greater Europe is, arguably, the second-most historic food safety event ever, second only to the 1993 *E. coli* O157:H7 Jack-in-the-Box outbreak. The reasons for this include the pathogen itself, the scope of the outbreak, the illnesses and impacted populations, the food vehicle, the news media coverage, and the unpredictable nature of the outbreak. Akin to the way scholars of terrorism view 9/11 as the landmark “unknown unknown” in global security studies, food safety scholars will view the 2011 German outbreak as a momentous occasion. If one argues that anticipatory thinking and a respect for unknown unknowns is important for food safety policy, then using the German outbreak as a case study both relevant and prudent. Of course, the very challenge with unknown unknowns is their elusiveness; yet, the more we try to uncover patterns and learn lessons from past mistakes, the more likely we are to steadily turn the unknowns into knowns. While there are multiple *strategies* advocated for in this chapter, *lessons* are also enumerated in this section for organization and simplicity. Such an approach is not unusual: modern-day influenza researchers often reference past influenzas (*e.g.*, the 1918 influenza pandemic) to learn from the past and anticipate future flus.

## 7.2.2. Lessons and questions from the German *E. coli* O104:H4 outbreak

### 7.2.2.1. Lesson 1: Look for what you *think you know*, but also look for other characteristics that may prove valuable later.

In their summative report<sup>302</sup> on the outbreak, epidemiologists at Germany's Robert Koch Institute state that the outbreak began in May 2011 with the onset of diarrheal symptoms in patients. In the affected, diarrhea often progressed to bloody diarrhea and hemolytic uremic syndrome (HUS). Cases peaked on May 22, with sporadic illnesses reported until the outbreak was declared over on July 26. The outbreak was massive: in total, there were 855<sup>303</sup> cases of HUS and 2,987 cases of acute gastroenteritis; 35 HUS patients and 18 EHEC gastroenteritis patients died. Women were more frequently diagnosed with bloody diarrhea and HUS, and overall, the majority of cases were adults, unusual for an EHEC or STEC event. The implicated vehicle was sprouts.<sup>304,305</sup> The number of cases per country is given in Table 7-1.

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<sup>302</sup> Robert Koch Institute, "Report: Final Presentation and Evaluation of Epidemiological Findings in the EHEC O104:H4 Outbreak, Germany 2011.," (Berlin2011).

<sup>303</sup> For example, in Germany there were 696 cases of HUS reported for the entire period of 2001-2010

<sup>304</sup> Robert Koch Institute.

<sup>305</sup> F Scheutz et al., "Characteristics of the Enterohemorrhagic Shiga Toxin/Verotoxin-Producing *Escherichia coli* O104:H4 Strain Causing the Outbreak of Haemolytic Uraemic Syndrome in Germany, May to June 2011," *Euro surveill* 16, no. 24 (2011).

**Table 7-1: Number of cases in the 2011 German sprouts STEC O104 outbreak<sup>306</sup>**

| <b>Country</b> | <b>EHEC cases</b> | <b>HUS cases</b> | <b>Deaths</b> |
|----------------|-------------------|------------------|---------------|
| Germany        | 2,947             | 818              | 51            |
| Sweden         | 35                | 18               | 1             |
| Denmark        | 16                | 10               | 0             |
| Netherlands    | 7                 | 4                | 0             |
| Switzerland    | 5                 | 0                | 0             |
| Austria        | 4                 | 1                | 0             |
| France         | 5                 | 9                | 0             |
| United Kingdom | 3                 | 4                | 0             |
| United States  | 2                 | 4                | 1             |
| Canada         | 1                 | 0                | 0             |
| Czech Republic | 1                 | 0                | 0             |
| Greece         | 1                 | 0                | 0             |
| Luxembourg     | 1                 | 1                | 0             |
| Norway         | 1                 | 0                | 0             |
| Poland         | 1                 | 2                | 0             |
| Spain          | 1                 | 1                | 0             |
| <b>Total</b>   | <b>2,987</b>      | <b>855</b>       | <b>53</b>     |

As the outbreak began and progressed, scientists were faced with identifying the causative agent, and, in an effort to do so, were wise in performing several different analyses. First, stool samples were enriched and plated on extended-spectrum- $\beta$ -lactamase (ESBL) agar plates, cefixime-tellurite sorbitol MacConkey agar plates (CT-SMAC), and screened by polymerase chain reaction (PCR) for important STEC-related genes: *stx*, *eae*, and EHEC-*hlyA*, (genes commonly associated with STEC outbreaks). Other typical molecular features were analyzed as well to create a virulence profile to compare with other outbreak samples.<sup>307</sup> New isolates were screened for the typical STEC-related virulence characteristics, but were also tested for virulence loci typical for EPEC, EIEC, and EAEC. Screening for the other *E. coli* characteristics was crucial.

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<sup>306</sup> Robert Koch Institute.

<sup>307</sup> Martina Bielaszewska et al., "Characterisation of the *Escherichia coli* Strain Associated with an Outbreak of Haemolytic Uraemic Syndrome in Germany, 2011: A Microbiological Study," *The Lancet Infectious Diseases* 11, no. 9 (2011).

#### 7.2.2.2. Lesson 2: Move quickly to assemble evidence.

Soon, the researchers had analyzed eighty samples, and the results were clear: this was a heretofore unseen bacteria with various, critical characteristics. All isolates possessed genes for the O104 somatic and H4 flagellar antigen; thus, one serotype was involved in the outbreak. Similar to STEC, the isolates produced the *stx<sub>2</sub>* gene (encodes production of Shiga toxin), produced typical STEC genes for adhesion, and possessed the ESBL phenotype. On the other hand, the isolates lacked certain STEC characteristics, most notably the *eae* gene that encodes intimin and genes for hemolysin production. The aforementioned analysis for EAEC characteristics yielded interesting results: O104 produced the EAEC-associated virulence plasmid pAA and the *aggA* gene that gives EAEC their unique adherence pattern in intestinal cells.<sup>308</sup> The use of an Early Warning Response System by the Robert Koch Institute (RKI), alongside collaborations with the World Health Organization Collaborating Centre (WHO CC), the Statens Serum Institut (Denmark; via the Danish *E. coli* network), and postings on the Urgent Inquiry Network (UIN) and Epidemic Intelligence Information System (EPIS) by the Food- and Waterborne Diseases and Zoonoses (FWD) Surveillance Network of the European Centre for Disease Control and Prevention (ECDC), helped in the quick assembly of evidence.<sup>309</sup> After the outbreak, public health authorities stated that “shortening the interval to diagnosis and identification of the source of infection is critical” to control *E. coli* O104:H4. Indeed, the rapid movement by those in charge was important.<sup>310</sup>

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<sup>308</sup> Ibid.

<sup>309</sup> Scheutz et al. Also involved were the European Union Reference Laboratory for *E. coli* (EU-RL), the Global Food-borne Infections Network (GFN), WHO offices, and PulseNet offices of the U.S. CDC.

<sup>310</sup> Helge Karch et al., "The Enemy within Us: Lessons from the 2011 European *Escherichia coli* O104:H4 Outbreak," *EMBO Molecular Medicine* 4, no. 9 (2012), p. 841.

### 7.2.2.3. Lesson 3: Shift unknown unknowns to unknown knowns and known knowns.

Present in this outbreak was a unique *E. coli* that had phenotypical characteristics aligning with both STEC and EAEC. Scientists had discovered *E. coli* O104:H4: a bacterium “unprecedented among enteric pathogens in its virulence.”<sup>311</sup> What was now known is that the presence of certain genes (such as *gnd* and *stx*<sub>2</sub>, among others previously mentioned), tellurite resistance, and the H4 antigen made this a unique bacterium.<sup>312,313</sup> The bacterium was a strong biofilm former and agglutinated well with human red blood cells.<sup>314</sup> Scientists were able to compare this and other information to previously isolated *E. coli* across Germany and the world, discovering this O104:H4 indeed matched a reference strain from 2001<sup>315</sup> but was unique otherwise to other STEC and EAEC. The value of strain collections and information sharing helped epidemiologists and scientists alike disseminate the necessary information about the outbreak.

There were still plenty of unknowns to shift to knowns. The first was source attribution and natural reservoirs of *E. coli* O104:H4. Initially, scientists believed that cattle were not the reservoir, due to the non-isolation of O104:H4-related genes (or any EAEC genes for that matter) in fecal samples from farms in the outbreak region of northern Germany.<sup>316</sup> However, an analysis by Cabal et al. (2015) pooled 970 cattle fecal samples from Germany and Spain and analyzed them by PCR for nine virulence-associated genes (VAGs), including those related to O104:H4,

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<sup>311</sup> Bielaszewska et al., p. 675.

<sup>312</sup> Lothar H. Wieler et al., "No Evidence of the Shiga Toxin-Producing *E. coli* O104:H4 Outbreak Strain or Enterohaggative *E. coli* (Eaec) Found in Cattle Faeces in Northern Germany, the Hotspot of the 2011 Hus Outbreak Area," *Gut Pathogens* 3, no. 1 (2011).

<sup>313</sup> Bielaszewska et al.

<sup>314</sup> Scheutz et al.

<sup>315</sup> While both the 2001 strain and the 2011 strain are O104:H4, they differ in plasmid content and fimbrial genes. For more, see: Mellmann et al.

<sup>316</sup> Wieler et al.

and found that six samples from a German slaughterhouse near the epicenter of the outbreak were positive for genes encoding Stx2, the aggregative mechanisms, the O104 antigen, and the H4 antigen, implying a bovine reservoir is at least possible.<sup>317</sup> Some surmised that cucumbers, tomatoes, or leafy salads were responsible for the outbreak.<sup>318</sup> The actual source of the outbreak was contaminated fenugreek sprouts. The seeds were imported from Egypt into Rotterdam, distributed to Germany, and then partially distributed in the UK and France.<sup>319</sup> The global nature of food highlights the risk of outbreaks (and the bacteria causing them) easily spreading. Furthermore, the risk associated with sprouts, discussed several times in this dissertation, is underscored yet again: the growing conditions for sprouts are ideal for proliferation of pathogenic bacteria.<sup>320</sup>

#### **7.2.2.4. Lesson 4: Use new knowledge and question-asking to uncover more unknown unknowns.**

Shifting knowns to unknowns unveils where the next unknown unknown may be lurking. As a means of highlighting this phenomenon, the following italicized questions in this section serve as examples of the new unknown unknowns. Also proposed are the means in which current knowledge being revealed about *E. coli* O104:H4 may help answer those questions.

*How can scientists integrate the similar and dissimilar behaviors of STEC O157 and E. coli O104 to prevent either/both from being a problem in the food system?* In a research paper by Bohnlein et al. (2016) *E. coli* O104:H4 was able to survive in conditions similar to fermented

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<sup>317</sup> Adriana Cabal et al., "Detection of Virulence-Associated Genes Characteristic of Intestinal *Escherichia coli* Pathotypes, Including the Enterohemorrhagic/Enterotoxigenic O104:H4, in Bovines from Germany and Spain," *Microbiology and Immunology* 59, no. 8 (2015).

<sup>318</sup> Lothar Beutin and Annett Martin, "Outbreak of Shiga Toxin–Producing *Escherichia coli* (Stec) O104:H4 Infection in Germany Causes a Paradigm Shift with Regard to Human Pathogenicity of Stec Strains," *Journal of Food Protection* 75, no. 2 (2012).

<sup>319</sup> Karch et al.

<sup>320</sup> Beutin and Martin.

sausages, at times out performing *E. coli* O157:H7.<sup>321</sup> Additionally, it was determined that *E. coli* O104:H4 secretes a bacteriocin that may aid in survival and persistence.<sup>322</sup>

*How can the adaptation to other environments or niches help food microbiologists understand future food safety challenges?* The genomes of two U.S.-isolated strains of STEC O104 were analyzed by shotgun sequencing, and similarity between the two strains (O104:H21 and O104:H7) were more similar to each other than the German outbreak strain and O104 strains isolated from Africa.<sup>323</sup> The differences between the German strain, African strains, and these strains is likely due to gain and loss of mobile genetic elements during evolution, and the authors suggest “that genetic variation of STEC O104 may be partly due to adaptation to local environments and interactions with other bacteria and hosts.”<sup>324</sup> This could mean that there are likely other O104 strains circulating, and even some similarly virulent O104:H4 strains that may be genetically different due to the external environment.<sup>325</sup> The right niche could lead to a competitive advantage for an emerging pathogen, leading to an explosion in population and circulation.

*How can the “potential” for something to happen (i.e., gaining new genetic elements) help regulators forecast?* The United States regulates six serogroups of STEC: O26, O45, O103, O111, O121, O145, and O157:H7.<sup>326</sup> However, numerous strains of human STEC have been isolated across the globe, strains with differing levels of virulence and pathogenicity. For

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<sup>321</sup> Christina Böhnlein et al., "Fitness of Enterohemorrhagic *Escherichia coli* (EHEC)/Enterobacteriaceae *E. coli* O104:H4 in Comparison to That of EHEC O157: Survival Studies in Food and *in vitro*," *Applied and Environmental Microbiology* 82, no. 21 (2016).

<sup>322</sup> *Ibid.*

<sup>323</sup> Xianghe Yan et al., "Genome Sequencing and Comparative Genomics Provides Insights on the Evolutionary Dynamics and Pathogenic Potential of Different H-Serotypes of Shiga Toxin-Producing *Escherichia coli* O104," *BMC Microbiology* 15, no. 1 (2015).

<sup>324</sup> *Ibid.*, pp. 16-17.

<sup>325</sup> *Ibid.*

<sup>326</sup> "Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products," *Federal Register* 76 (20 September 2011): 58157-59

example, Beutin et al. (2004) isolated 677 STEC strains over a three-year period in Germany, analyzing by types of Shiga toxins present, genetic profiles (including *eae* and hemolysin), O and H antigen serotyping, and clinical signs.<sup>327</sup> In that study, eleven serotypes accounted for 69% of all STEC strains (the U.S.-regulated O157, O26, O103, O111, and O145 and the nonregulated O91, O128, O113, O146, O118, and O76).<sup>328</sup> Further, 41 STEC strains belonging to 31 serotypes were identified, revealing the wide array of STEC able to colonize humans.<sup>329</sup> A survey of bovine fecal and soil samples from farms in Ireland revealed the presence of 107 STEC isolates representing 18 serotypes, including the U.S.-regulated O26:H11 and O145:H28.<sup>330</sup> Interestingly, strains O2:H27, O13/O15:H2, and ONT:H27 were also isolated, and because they carry *stx*<sub>1</sub>, *stx*<sub>2</sub>, and *eaeA*, may emerge as threats to public health.<sup>331</sup> A similar Argentine<sup>332</sup> study, this using multilocus sequence typing (MLST), identified 59 STEC isolates from 31 serotypes, including 17 novel sequence types.<sup>333</sup> The increasing amount of beef imports to the United States, including those from places like the EU and South America (*i.e.*, the nations in the aforementioned studies) increases the likelihood that these emerging STEC strains may enter the food chain and impact human health. Or, new *E. coli* strains of any category (Table 1-2) could enter the food chain and begin exchanging elements. Finally, in the U.S., 940 presumed non-O157 STEC isolates were sent to CDC between 1983 and 2002. The most common serogroups were the “Big Six”; however, also isolated was O165, containing *stx*<sub>1</sub>, *stx*<sub>2</sub>, and *eae*, as well as

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<sup>327</sup> Lothar Beutin et al., "Characterization of Shiga Toxin-Producing *Escherichia coli* Strains Isolated from Human Patients in Germany over a 3-Year Period," *Journal of Clinical Microbiology* 42, no. 3 (2004).

<sup>328</sup> *Ibid.*

<sup>329</sup> *Ibid.*

<sup>330</sup> Áine Monaghan et al., "Serotypes and Virulence Profiles of Non-O157 Shiga Toxin-Producing *Escherichia coli* Isolates from Bovine Farms," *Applied and Environmental Microbiology* 77, no. 24 (2011).

<sup>331</sup> *Ibid.*

<sup>332</sup> Interestingly, Argentina has the highest incidence of HUS in the world, according to Marta Rivas et al., "Diarrheagenic *Escherichia coli* in Argentina," *Pathogenic Escherichia coli in Latin America* 1 (2010).

<sup>333</sup> Jimena S. Cadona et al., "Genetic Relatedness and Novel Sequence Types of Non-O157 Shiga Toxin-Producing *Escherichia coli* Strains Isolated in Argentina," *Frontiers in Cellular and Infection Microbiology* 6, no. 93 (2016).

several other O-groups (although not possessing the riskier *stx*<sub>2</sub> or *eae*).<sup>334</sup> While the other strains (other n=139; undetermined n=123) did not possess the virulence combinations as the “Big Six”, they may serve as candidates for a similar phenomenon as the hybridization seen in the German O104 outbreak. U.S.-regulators should pay attention to STEC O55:H7 as well. This strain caused an outbreak in 31 persons (13 developed HUS) in England. This strain had “parallel, convergent evolutionary history” with STEC O157:H7, indicating “a common driver in the evolutionary process.”<sup>335</sup> This pathogen, highly pathogenic but not previously isolated in humans or animals in the UK, harbors Stx2a, the same toxin in STEC O157:H7, which should concern regulators. From an unknown unknown perspective, the advent of STEC O55:H7 signals a common driver in the evolution of it at O157:H7, demonstrating “adaptation to a new niche” possibly “accompanied by modification of gene expression” that could (and likely will) occur again in the future.<sup>336,337</sup> Another serogroup, STEC O91, has caused severe infections and HUS, and has been isolated from food, animals, and the environment.<sup>338</sup> Differing O91 strains have lacked certain LEE-island-encoded genes or the entire LEE-island itself, but still can cause disease while having “genetic room” for additional virulence genes.<sup>339</sup> Insights from the evolution of various

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<sup>334</sup> John T. Brooks et al., "Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in the United States, 1983–2002," *The Journal of Infectious Diseases* 192, no. 8 (2005).

<sup>335</sup> Kyle Schutz et al., "Evolutionary Context of Non–Sorbitol-Fermenting Shiga Toxin–Producing *Escherichia coli* O55:H7," *Emerging Infectious Diseases* 23, no. 12 (2017). Interestingly, the authors posit that the pathogen may have arrived from Ireland by transit on migratory birds. Birds have also been thought to spread antimicrobial resistant elements at CAFOs. See: James C. Carlson et al., "Mechanisms of Antimicrobial Resistant *Salmonella Enterica* Transmission Associated with Starling–Livestock Interactions," *Veterinary Microbiology* 179, no. 1–2 (2015).

<sup>336</sup> Schutz et al., p. 1962.

<sup>337</sup> Anthony T. Maurelli, "Black Holes, Antivirulence Genes, and Gene Inactivation in the Evolution of Bacterial Pathogens," *FEMS Microbiology Letters* 267, no. 1 (2007).

<sup>338</sup> Peter C. H. Feng et al., "Shiga Toxin-Producing Serogroup O91 *Escherichia coli* Strains Isolated from Food and Environmental Samples," *Applied and Environmental Microbiology* 83, no. 18 (2017).

<sup>339</sup> Ibid.

*E. coli* strains should be better understood to anticipate and more quickly identify new STECs as they appear.<sup>340</sup>

*How can commonly-used tools (e.g., PCR) be deployed for surveillance and early detection of threats?* Scientists have developed a PCR-based subtyping method to distinguish relatedness of various Stx toxins and *stx* genes, resulting in a three-tiered nomenclature system to better describe strains based upon cytotoxin type, subtype, and variant.<sup>341</sup> This information will glean light on the outcomes associated with various Shiga toxins and the STEC that carry them. Such knowledge will assist in comparison of strains during an outbreak and will permit stronger risk assessment and outbreak detection protocols. Additionally, the previously survey of O104:H4 genes in pooled bovine fecal samples demonstrated that conducting PCR for such genes in surveillance studies “could be an efficient early-warning tool for the emergence of zoonotic *E. coli* in livestock.”<sup>342</sup>

*How will continued genomic and molecular approaches help unveil the mechanisms of current pathogens and give insight to future pathogens and associated virulence?* When pathogens use virulence factors, they are delivered to host cells as free proteins that interact with receptors on the cell surface, are injected by molecular syringes like the T3SS, or are associated with outer membrane vesicles (OMVs), “spherical, bilayered nanostructures” that “enable a direct, simultaneous, and coordinated delivery of virulence factors” alongside antimicrobial and immunomodulatory compounds.<sup>343</sup> Work by Kunsmann (2015) has demonstrated that *E. coli*

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<sup>340</sup> Vinicius Silva Castro et al., “Shiga-Toxin Producing *Escherichia coli*: Pathogenicity, Supershedding, Diagnostic Methods, Occurrence, and Foodborne Outbreaks,” *Comprehensive Reviews in Food Science and Food Safety* 16, no. 6 (2017).

<sup>341</sup> Flemming Scheutz et al., “Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature,” *Journal of Clinical Microbiology* 50, no. 9 (2012).

<sup>342</sup> Cabal et al., p. 433

<sup>343</sup> Lisa Kunsmann et al., “Virulence from Vesicles: Novel Mechanisms of Host Cell Injury by *Escherichia coli* O104:H4 Outbreak Strain,” *Scientific Reports* 5 (2015), p.2.

O104:H4 OMVs “bind to and are internalized by human intestinal epithelial cells, deliver the OMV-associated virulence factors intracellularly, and induce...apoptosis and an inflammatory response.”<sup>344</sup> This is similar to OMV-delivery seen in *E. coli* O157:H7. Scientists and regulators should continue to explore OMVs and gain insight to how their delivery (and their control) may impact future interventions. Researchers investigated the role of extracellular proteins in their interactions with the environment: proteins released during growth of *E. coli* O157:H7 in minimal media were for extracellular (signaling proteins or attachment) while those released by *E. coli* O104:H4 had functions within the cytoplasm of the host cell.<sup>345</sup> In part one of the dissertation, we discussed the impact of stress on STEC; here, researchers demonstrate that O104:H4 responds differently to stress than O157:H7.<sup>346</sup> Islam et al. (2016) “hypothesize that the release of cell contents through porins or OMVs may significantly contribute to the localized accumulation of extracellular proteins and nucleic acids, which promote the initiation of biofilm formation.”<sup>347</sup>

### 7.2.3. Conclusion.

The *E. coli* O104:H4 strain displayed an augmented virulence, likely due to its enhanced adherence capability, the presence of antibiotic resistance plasmid, and the presence of Stx2.<sup>348</sup> What should also concern public health officials (and be used as a means to anticipate other outbreaks) is “that LEE and the *nle* genes present in classical EHEC strains can be substituted by

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<sup>344</sup> Ibid., p. 11.

<sup>345</sup> Nazrul Islam et al., "Comparative Proteomic Analysis Indicates That Extracellular Proteins Released by *Escherichia coli* O157:H7 and O104:H4 Have Different Cellular Origins and Functions," *Applied and Environmental Microbiology* (2016).

<sup>346</sup> Ibid.

<sup>347</sup> Ibid., pp. 17-18.

<sup>348</sup> Beutin and Martin.

EAEC plasmid-encoded aggregative adherence mechanisms to enable Stx2-producing EAHEC O104:H4 to cause hemorrhagic colitis and HUS.”

Perhaps a final, foreboding nod to the dominance that unknown unknowns will likely continue to exert, Karch et al. (2012) said the following about the outbreak:

*One of the many lessons of this outbreak is that E. coli has an exquisite mastery to acquire and combine genes that may covert the pathogen into an insidious one, able to subvert the physiology of human cells. It is not at all clear that humans are equally clever at counteracting or even detecting the bacteria and preventing its rapid spread.*<sup>349</sup>

## **7.3. IMMEDIATE STRATEGIES (NUMBERS 2 AND 3)**

### **7.3.1. Introduction.**

The events of and lessons learned from the 2011 German *E. coli* O104:H4 outbreak serve as a springboard for the pursuit of knowledge related to unknown unknowns in the food system. As a means to attain this goal, two immediate strategies are now proposed (strategies 2 and 3). These strategies should be implemented by public health officials, food microbiologists, regulators, academics, and the private sector to increase identification and response capability when the next unknown unknown occurs.

### **7.3.2. Strategy 2: Target food groups of high and/or increasing consumption.**

The foods consumed in America are tracked by a partnership of USDA’s Agricultural Research Service (ARS), and HHS.<sup>350</sup> When compiled, this report is known as “What We Eat In America”, an integration of USDA’s Continuing Survey of Food Intakes by Individuals (CSFII)

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<sup>349</sup> Karch et al., p. 847

<sup>350</sup> United States Department of Agriculture, "WWEIA/NHANES Overview," <https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweianhanes-overview/>.

and HHS's National Health and Nutrition Examination Summary (NHANES).<sup>351</sup> For strategy two, a thoughtful approach to forecasting the next food safety issue begins with *knowing what people are eating and understanding those foods that are increasing in popularity*. To do so, the use of data from CSFII, NHANES, and other surveys (e.g. Pew Research Studies, data from Centers for Science in the Public Interest [CSPI]), should be mined to understand from where the next threat may emanate.

For example, a major 2016 report from *JAMA* looked at trends in 34,000 adult eating habits based on seven surveys conducted between 1999 and 2012.<sup>352</sup> Trends show that consumption of sugary drinks is decreasing, while intake of whole grains, nuts, seeds, and legumes are increasing.<sup>353</sup> Consumption of fruits, vegetables, and processed meats did not change; fish and shellfish and egg consumption did increase.<sup>354</sup> Milk consumption has been decreasing, but consumption of cheese has increased.<sup>355</sup> A 2016 Pew Research Study on American food consumption revealed similar trends related to dairy: less milk and more cheese and yogurt.<sup>356</sup> Americans consume less sugar but much more corn-derived sweeteners, less red meat but more chicken (47.9 pounds per year), and more cooking oils than their 1970 counterparts.<sup>357</sup> The USDA is predicting record-breaking consumption of beef and poultry in 2018, with consumers eating a forecasted 222.2 pounds for the year<sup>358</sup>; the fact that USDA is actively involved in forecasting demonstrates how doable and valuable such a strategy is. Along

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<sup>351</sup> Ibid.

<sup>352</sup> C. D. Rehm et al., "Dietary Intake among U.S. Adults, 1999-2012," *JAMA* 315, no. 23 (2016).

<sup>353</sup> Ibid.

<sup>354</sup> Ibid.

<sup>355</sup> Ibid.

<sup>356</sup> Drew Desilver, "What's on Your Table? How America's Diet Has Changed over the Decades," Pew Research Center, <http://www.pewresearch.org/fact-tank/2016/12/13/whats-on-your-table-how-americas-diet-has-changed-over-the-decades/>.

<sup>357</sup> Ibid.

<sup>358</sup> Bloomberg, "'Beef Will Always Be King.' Americans Are Set to Eat More Meat in 2018 Than Ever Before," *Fortune*, <http://fortune.com/2018/01/02/meat-consumption-america-2018/>.

the same vein, USDA predicts that, by the end of 2018, pork production in America will equal that of beef, due to the increasing popularity of bacon and the growth of Asian and Latino cuisines that often include pork in dishes.<sup>359</sup> Americans are buying more beef “in the ground form—about 50 percent versus 42 percent a decade ago.”<sup>360</sup> Finally, consumption of organic foods continues to grow year over year, especially organic fruits and vegetables.<sup>361</sup>

How can these lessons be leveraged from a food safety/defense perspective? The increasing consumption of eggs and chicken likely means *Salmonella* will continue to be a problem for public health practitioners: in the U.S. and Canada, eggs account for 23.3% of nontyphoidal *Salmonella* infections, and poultry accounts for 22.6% (ranking first and second in accountability, respectively).<sup>362</sup> The percent of *Campylobacter* spp. illnesses attributed to poultry (49.6%) also has implications for increasing chicken consumption.<sup>363</sup> Recommendations for increased food and vegetable consumption may make some foodborne parasites (*e.g.* *Cryptosporidium* spp.) more prevalent.<sup>364</sup>

Increased consumption of fish and shellfish, as documented by the *JAMA* study,<sup>365</sup> suggests that this sector of the food industry might be attractive for an intentional or result in an unintentional contamination event: the U.S. imports the majority of the fish it consumes from all over the globe, opening up vulnerabilities from both improperly enforced HACCP standards as

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<sup>359</sup> Caitlin Dewey, "Why Americans Are Eating More Pork Now Than They Have in Decades," *The Washington Post* 2017.

<sup>360</sup> Anonymous, "How Safe Is Your Beef?," *Consumer Reports* 2015.

<sup>361</sup> Kristen Leigh Painter, "Americans Are Eating More Organic Food Than Ever, Study Finds," *Star Tribune* 2017.

<sup>362</sup> Sandra Hoffmann et al., "Attribution of Global Foodborne Disease to Specific Foods: Findings from a World Health Organization Structured Expert Elicitation," *PLoS ONE* 12, no. 9 (2017).

<sup>363</sup> *Ibid.*

<sup>364</sup> *Ibid.*

<sup>365</sup> Rehm et al.

well as the presence of unsavory actors abroad.<sup>366,367</sup> For example, in the fall of 2017 it was revealed that North Korean citizens were preparing seafood for U.S. restaurants and retail stores through a work-partnership with China; implicated products were being sold in American Walmart and Aldi grocery stores.<sup>368</sup> Additionally, fish is often implicated in economically motivated adulteration as inferior species are substituted for more profitable species.<sup>369</sup>

Increasing consumption of chicken, compared to its beef and pork counterparts, may lead to an increase in the circulation of antimicrobial resistance (AMR) genes.<sup>370</sup> Chicken, produced in cramped chicken houses that breed AMR pathogens are shipped daily across the continent and the globe.<sup>371</sup> A study found that 41% of chicken sampled from a grocery store contained AMR *Staphylococcus aureus*.<sup>372</sup> In another study, a high prevalence of ESBL-producing *E. coli* was found in chicken meat samples in France despite reduced use of antimicrobials in the EU.<sup>373</sup>

The increased consumption of organic produce may lead to the proliferation of pathogenic bacteria that are not controlled by certain interventions; however, this has yet to occur, according to the journal *Sustainable Agriculture*.<sup>374</sup> Nonuse of traditional, field-applied

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<sup>366</sup> Sven M. Anders and Julie A. Caswell, "Standards as Barriers Versus Standards as Catalysts: Assessing the Impact of HACCP Implementation on U.S. Seafood Imports," *American Journal of Agricultural Economics* 91, no. 2 (2009).

<sup>367</sup> Laurian J. Unnevehr, "Food Safety Issues and Fresh Food Product Exports from LDCs," *Agricultural Economics* 23, no. 3 (2000).

<sup>368</sup> Tim Sullivan, Hyung-Jin Kim, and Martha Mendoza, "North Koreans Prepping Seafood for U.S. Stores, Restaurants," *USA Today* 2017.

<sup>369</sup> Personal communication with A.L. Nutsch. April 20, 2018.

<sup>370</sup> Vangelis Economou and Panagiota Gousia, "Agriculture and Food Animals as a Source of Antimicrobial-Resistant Bacteria," *Infection and Drug Resistance* 8 (2015).

<sup>371</sup> Ramanan Laxminarayan et al., "Antibiotic Resistance—the Need for Global Solutions," *The Lancet Infectious Diseases* 13, no. 12 (2013).

<sup>372</sup> Andrew E. Waters et al., "Multidrug-Resistant *Staphylococcus Aureus* in US Meat and Poultry," *Clinical Infectious Diseases* (2011).

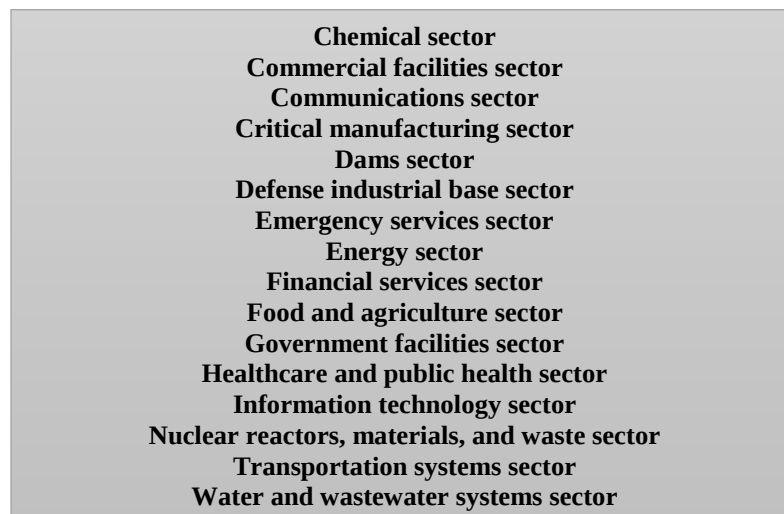
<sup>373</sup> Tiago Casella et al., "High Prevalence of ESBLs in Retail Chicken Meat Despite Reduced Use of Antimicrobials in Chicken Production, France," *International Journal of Food Microbiology* 257 (2017).

<sup>374</sup> Denis Lairon, "Nutritional Quality and Safety of Organic Food," in *Sustainable Agriculture Volume 2*, ed. Eric Lichtfouse, et al. (Dordrecht: Springer Netherlands, 2011).

interventions in organic production may permit the return of or increase in populations of plant pests, creating phytosanitary food supply issues.

### **7.3.3. Strategy 3: Assess FA threats primarily rooted in other critical infrastructures.**

DHS oversees sixteen critical infrastructure sectors. Most were first mandated by Homeland Security Presidential Directive 7, promulgated by the Bush administration in 2003.<sup>375</sup> This was superseded by the Obama administration's PPD 21, still in effect at this writing, to strengthen and maintain certain infrastructures (Figure 7.2, below) vital to the United States.<sup>376,377</sup>



**Figure 7.2: DHS Critical Infrastructures<sup>378</sup>**

The U.S. government's goal "is to minimize, with a limited amount of resources, the expected impact on the nation's critical infrastructure of any future terrorist attack."<sup>379</sup> Of course, the government's resources are finite, and allocation of resources must be prioritized. To achieve

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<sup>375</sup> Department of Homeland Security, "Homeland Security Presidential Directive 7: Critical Infrastructure Identification, Prioritization, and Protection," <https://www.dhs.gov/homeland-security-presidential-directive-7>.

<sup>376</sup> Obama.

<sup>377</sup> Department of Homeland Security, "Critical Infrastructure Sectors," <https://www.dhs.gov/critical-infrastructure-sectors>.

<sup>378</sup> Ibid.

<sup>379</sup> John Moteff, Claudia Copeland, and Josh Fischer, "Critical Infrastructures: What Makes an Infrastructure Critical?," (Congressional Research Service, 2003)., p.12.

this prioritization, lawmakers can identify vulnerabilities that are being birthed in another sector but impact FA.<sup>380</sup> Or, they can find where infrastructures are interdependent, relying on each other's vitality to persist.<sup>381</sup> The FA sector is very reliant upon the water sector (providing a supply of potable water and wastewater facilities), the transportation systems sector (transporting the inputs and outputs of FA), and the chemical sector (fertilizers, pesticides, food processing aids).<sup>382</sup>

Another strong example is the interdependency of the FA sector and the information technology (IT) sector. The overlap between the FA and IT sectors demonstrates the “need for coordinated and trusted interagency partnerships across all levels of government.”<sup>383</sup> This permits the sharing of information (*e.g.*, warnings on threats, asset vulnerabilities, and action plans) to better anticipate multi-sector risks.

Imagine the following scenarios contrived by Keeley and Landry (2017):

*As equipment manufactures and food production companies become more reliant on automated, interconnected processes and manufacturing, the potential of a hacker breaching the production process increases drastically... [A terrorist group] could infiltrates a production facility and manipulates the readouts for a pasteurizer designed to decrease the risk of foodborne illness. If undetected, the delivered, consumed product would gravely endanger consumer safety. Under-processed food could be the delivery vehicle for dangerous human pathogens such as E. coli O157:H7, Listeria monocytogenes, and Salmonella spp. This is especially true for canned goods, where inadequate processing would increase the risk of botulism poisoning....An increasingly common practice in the agricultural sector is a process known as food irradiation, a form, and source of radiation. Producers introduce radiation into their produce, to extend the shelf life of food. These radiating facilities are automated. Suppose a bad actor altered the algorithms,*

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<sup>380</sup> Ibid.

<sup>381</sup> Ibid.

<sup>382</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan."

<sup>383</sup> Jose M. Yusta, Gabriel J. Correa, and Roberto Lacal-Arántegui, "Methodologies and Applications for Critical Infrastructure Protection: State-of-the-Art," *Energy Policy* 39, no. 10 (2011), p. 6113.

*drastically spiking radiation levels? Vast swathes of produce and food stores could be irradiated to dangerous levels.*<sup>384</sup>

The food industry is highly reliant upon IT, especially when it comes to data logging, machine calibration, and other critical processes that occur numerous times in food plants. Indeed, at the 10<sup>th</sup> Anniversary Homeland Defense/Security Education Summit at George Mason University, speakers time and again spoke about cross-sector threats.<sup>385</sup>

For both an FA and IT infrastructure crisis (and likely for the other fourteen critical infrastructures), sharing of information and cross-infrastructure coordination will be paramount. Communication challenges for both fields include “competing pressures faced by corporate executives which include meeting global market demands, managing risks to their enterprise, protecting trade secrets and proprietary information, and limiting corporate and shareholder exposure to legal liabilities.”<sup>386</sup> Nevertheless, both the European Commission and DHS (by way of the National Infrastructure Protection Plan) have encourage public-private cooperation (an essential contributor to resilience we mentioned in chapter 6) and information sharing as a means of infrastructure protection.<sup>387</sup> As Yusta et al. (2011) state: “protecting the interconnected and interdependent infrastructure requires a robust public-private partnership.”<sup>388</sup>

The DHS FA Sector Specific Plan explicitly states the role of IT in food protection:

*Cyber threats and attack tools evolve rapidly as the cyberattacking community shows ingenuity. Most attacks can be blocked by continuously updated computer security programs. Such programs involve adherence to procedural safeguards to the system; an effective, continuously adaptive firewall; the application of intrusion detection and intrusion prevention systems for detecting, reporting, and preventing external threats to the network and*

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<sup>384</sup> Keeley and Landry.

<sup>385</sup> Author was in attendance at this summit.

<sup>386</sup> Yusta, Correa, and Lacal-Arántegui., p. 6112.

<sup>387</sup> Ibid.

<sup>388</sup> Ibid., p. 6113.

*information systems; surveillance programs for detecting insider threats; the continuous training of system users on proper security procedures; use of passwords resistant to hacker compromise...*<sup>389</sup>

FA has grown dependent upon IT: “Large amounts of data are needed to drive the food manufacturing supply chain, from raw materials sourcing to plant production to end consumers.”<sup>390</sup> Furthermore, many companies in FA use Industrial Control Systems (ICS), which are becoming more connected. The vulnerability of this systems need to be better understood, as does the other technologies upon which the sector is very reliant.<sup>391</sup> Indeed, technology’s influence on the food system is only going to grow, with new technologies delivering solutions for producers and processors via “advanced sensors technology, the Internet of Things (IoT); cloud computing, ubiquitous geographic information system (GIS) technology, and powerful new platforms for integration and analytics.”<sup>392</sup>

#### **7.3.4. Policy ways forward.**

Regarding strategy 2, a process to obtain more up-to-date demographic monitoring tools like CSFII and NHANES databases should be implemented. Such data can be used to “watch” for food vehicles of increasing consumer importance. For strategy 3, other critical infrastructure have overlaps with the food system and face similar challenges to their security and resilience; collaboration among these infrastructures should continue.<sup>393</sup> The continued convening inter-sector meetings of the different sector-specific groups mandated in PPD 21 is encouraged. More communication and information sharing will strengthen each sector individually.

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<sup>389</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan."

<sup>390</sup> National Institute of Standards and Technology, "Agri-Food Manufacturing System and Supply Chain Integration," <https://www.nist.gov/programs-projects/agri-food-manufacturing-system-and-supply-chain-integration>.

<sup>391</sup> Department of Homeland Security, "Food and Agriculture Sector-Specific Plan."

<sup>392</sup> National Institute of Standards and Technology.

<sup>393</sup> Obama.

## 7.4. STRATEGY 4: BORROW CONCEPTS AND PRINCIPLES FROM METEOROLOGICAL FORECASTING

### 7.4.1. Introduction.

Meteorology, and specifically, weather forecasting, offers a unique perspective on predicting the next food safety-related “unknown unknown.” Meteorologists must integrate a wide array of variables from differing platforms (*e.g.*, radar data, satellite data, weather balloons, and general observations) as well as data-based input from computers to arrive at a forecast.<sup>394</sup> Weather forecasting involves “solving daily a system of nonlinear differential equations at about half a billion points per time step between the initial time and weeks to months ahead, and accounting for dynamic, thermodynamic, radiative and chemical processes working on scales from hundreds of meters to thousands of kilometers and from seconds to weeks.”<sup>395</sup> When dealing with time scales relating to hours, days, and weeks, a process known as numerical weather prediction (NWP) is used.<sup>396</sup> This process “involves representing the current atmospheric state on a three-dimensional grid, applying the physical and dynamic equations that govern how the atmosphere will change in time at each grid point, and repeating this process to generate a forecast of desired length.”<sup>397</sup>

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<sup>394</sup> American Meteorological Society, “Weather Analysis and Forecasting,” <https://www.ametsoc.org/ams/index.cfm/about-ams/ams-statements/statements-of-the-ams-in-force/weather-analysis-and-forecasting/>.

<sup>395</sup> Peter Bauer, Alan Thorpe, and Gilbert Brunet, “The Quiet Revolution of Numerical Weather Prediction,” *Nature* 525 (2015), p. 47.

<sup>396</sup> American Meteorological Society.

<sup>397</sup> *Ibid.*

#### 7.4.2. Using meteorological principles in food safety anticipatory thinking.

While predicting the weather may seem unrelated to food safety policymaking, in many ways, the similarities are striking. First, meteorology's goal is an accurate weather forecast. Put another way, application of scientific knowledge should result in predicting an experiment's outcome.<sup>398</sup> Similarly, food regulators seek some confidence that the means to control challenges (*e.g.*, implementing new standards for the control of STEC) will result in the predicted outcomes (fewer STEC outbreaks). The second similarity between weather prediction and the goals of a food safety regulator is that, for weather prediction, "accurate forecasts save lives, support emergency management and mitigation of impacts and prevent economic losses from high-impact weather."<sup>399</sup> Accurate predictions of food safety crises, and having the infrastructure and communication streams to correct a crisis afford the same things: lives saved, fewer economic losses, and a more confident public. Third, in the weather world as in the food safety world, the *extreme* events are most visible to the public (and the media). Millions eat food every day without issue, but a major outbreak in a commonly consumed product makes headlines in *USA Today* and on CNN. Fourth, forecasting weather—like anticipating food safety issues—is a "battleground, with the forces of predictability pitched against those of unpredictability."<sup>400</sup>

So, how does weather prediction go about solving the unpredictability problem? NWP models begin with using the tenants of thermodynamics, the ideal gas law, actual weather measurements (*e.g.*, wind speed, jet stream) and ***parameterization*** of unresolved values (*e.g.*, long- and short-wave radiation, deep and shallow convections, orographic drag, and atmospheric chemistry).<sup>401</sup> Once these factors are determined, a process known as ensemble modeling can

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<sup>398</sup> Bauer, Thorpe, and Brunet.

<sup>399</sup> *Ibid.*, p. 47.

<sup>400</sup> *Ibid.*, p. 49.

<sup>401</sup> *Ibid.*

occur. In **ensemble forecasting**, “multiple (typically between 5 and 100) runs of numerical weather prediction models, which differ in the initial conditions and/or the numerical representation of the atmosphere” are performed by supercomputers to assemble a likely forecast for the next 12 hours to the next 12 days while reducing uncertainty.<sup>402</sup> Ensemble modeling helps account for the chaotic nature of the atmosphere while controlling for error; put another way, “very similar initial states of the atmosphere will sometimes become very different in time.”<sup>403</sup> It has been the increasing knowledge about physical processes, coupled with numerical development and advances in computing, that has advanced weather prediction.<sup>404</sup> Advances in knowledge about the environmental constraints facing bacteria, as well as the ways in which physical constraints (*e.g.*, temperature, pressure, salt, et cetera) control or permit their growth, could be inserted into a modeling system to better predict the survival and proliferation of bacteria. In fact, we propose that tools such as **parameterization** and **ensemble forecasting** could be used in predictive food microbiology to better ideate, predict, and respond to food safety issues.

Small changes in the parameters used for modeling can result in varied outcomes. This is highlighted in the dissonance between the U.S. and European weather prediction tools. In meteorological circles, the European Centre for Medium-Range Weather Forecasts (ECMWF) is viewed as superior to the United States’ Global Forecast System (GFS).<sup>405</sup> Insights in the systems show why. The ECMWF “uses a mathematical method called four-dimensional variational data assimilation, or 4D-Var,” where observations are not taken at a single point but

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<sup>402</sup> Tilmann Gneiting and Adrian E. Raftery, "Weather Forecasting with Ensemble Methods," *Science* 310, no. 5746 (2005), p. 248.

<sup>403</sup> American Meteorological Society.

<sup>404</sup> Gneiting and Raftery.

<sup>405</sup> David Kramer, "Europeans Shine in Weather Forecasting," *Physics Today* 69, no. 1 (2016).

over hours.<sup>406</sup> The American system is stuck in 3D (the fourth dimension in the European model is time), meaning the model is stuck in time, while the European model can move forwards and backwards across time. ECMWF trumps GFS in another way as well, when, during ensemble modeling forecasting, the European system performs fifty iterations of its forecasts compared to the U.S.'s twenty iterations.<sup>407</sup> Doing so reduces the spread of the data and increases confidence in the forecast. Policymakers must understand the power of adding more data and computing power into a model, be it a weather system as described here or a tool to manage food safety crises as we propose.

Food scientists and regulators ought to monitor the discipline of meteorology's advancements. This includes work by researchers on developing a "hybrid approach that combines discriminatively trained predictive models with a deep neural network that models the joint statistics of a set of weather-related variables."<sup>408</sup> This new modeling permits analysis of multiple variables at once, inputting historical data and local inferences to create a co-variance matrix, or kernel, giving smoothness to the model.<sup>409</sup> In a more simple way, if meteorologists (and in this case, computer engineers and statisticians) develop models that permit the input of more variables at one time, and can make those models smoother and granular, then the lessons learned in that scientific field may be applicable to the challenges faced in food safety. Indeed, food safety is a realm with multiple variables, historical data, and local conditions that could benefit from advanced computing. Further, it may be necessary to learn from the past.

Meteorologists, when modeling a NWP, apply statistical technique to correct systemic biases,

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<sup>406</sup> Ibid., p. 22

<sup>407</sup> Ibid.

<sup>408</sup> Aditya Grover, Ashish Kapoor, and Eric Horvitz, "A Deep Hybrid Model for Weather Forecasting," in *Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining* (Sydney, NSW, Australia: ACM, 2015).

<sup>409</sup> Ibid.

often through comparisons of past model forecasts with actual outcomes, making the model itself stronger.<sup>410</sup>

Meteorology has already been used to inform risk communication in other realms. For example, policy researchers Monahan and Steadman (1996) draw parallels between meteorology and mental health, advocating that meteorological lessons (forecasting rare and severe events) can be applied to the mental health law issue of violence perpetrated by the mentally ill.<sup>411</sup> They reference a National Research Council's report *Improving Risk Communication* on why weather forecasters are proficient at their task: frequent practice, the availability of statistical data, feedback on results, and educational programming.<sup>412</sup> The same characteristics, developed and honed, could apply to food safety prediction and risk assessment. Indeed, meteorologists, mental health professionals, and food safety professionals all perform risk assessment in a similar way:

- (a) *Someone credentialed as a professional*
- (b) *assesses risk factors derived from past experience or from theories and*
- (c) *processes these risk factors with the aid of explicit or implicit prediction models.*
- (d) *The professional then constructs a likelihood estimate (or "forecast") of the event of interest occurring, and, finally,*
- (e) *the professional issues a risk communication containing this forecast to various audiences of relevant decision makers.*<sup>413</sup>

#### **7.4.3. Real-world food application.**

Akin to the map-based weather forecasts we see on television every day, similar modeling and data display can be used in the realm of food microbiology. Tarr et al. (2018) used reported *E. coli* O157:H7 infections and phylogenetic characterization of the isolates to

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<sup>410</sup> American Meteorological Society.

<sup>411</sup> John Monahan and Henry J Steadman, "Violent Storms and Violent People: How Meteorology Can Inform Risk Communication in Mental Health Law," *American Psychologist* 51, no. 9 (1996).

<sup>412</sup> *Ibid.*

<sup>413</sup> *Ibid.*, p. 932.

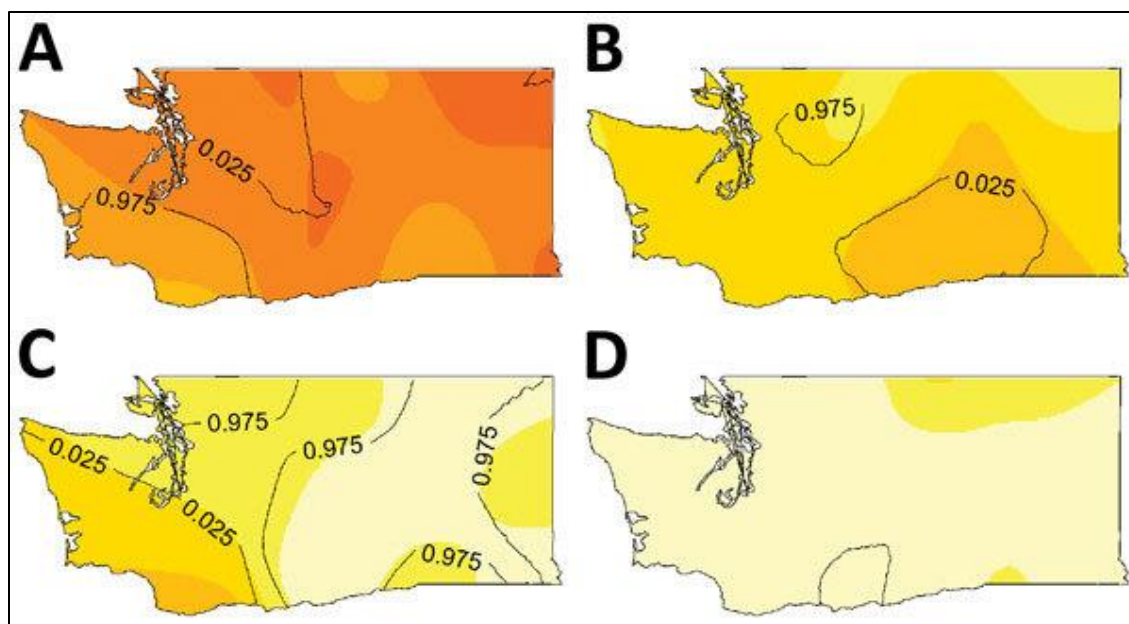
geographically segregate specific lineages in Washington state.<sup>414</sup> Following lineage analysis, the authors “estimated a smoothed probability surface for each lineage by comparing the distance between cases infected with the same lineage to the distance between cases infected with different lineages” while spatially segregating by comparing probability surfaces with a consistent infection proportion (Figure 7.3).<sup>415</sup> According to the study’s result a “founder effect” was established, “in which an ancestral pathogen has become established in a region, persisted, and expanded and occasionally crosses into the human population”; this lineage-based knowledge “has the potential to focus both outbreak investigations and efforts to identify potential reservoirs.”<sup>416</sup> Similar to the way weather forecasters have used multiple models, data sources, and relevant scientific information, the scientists here have defined high probability regions for certain *E. coli* O157:H7 lineages. Instead of predicting rain and snow, they are predicting STEC.

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<sup>414</sup> Gillian A. M. Tarr et al., "Geogenomic Segregation and Temporal Trends of Human Pathogenic *Escherichia coli* O157:H7, Washington, USA, 2005–2014," *Emerging Infectious Diseases* 24, no. 1 (2018).

<sup>415</sup> Ibid., p. 33. The special segregation analyses also incorporated time.

<sup>416</sup> Ibid., p. 36-38.



**Figure 7.3: *Escherichia coli* O157:H7 lineage frequency among culture-confirmed human cases resported in Washington, USA, 2005-2014. A) Lineage Ib; B) lineage IIa; C) lineage IIb; D) rare lineages (12 different clinically rare lineages). Lineage-specific probability surfaces were determined by kernel-based estimation of spatial segregation. Darker shading indicates higher risk for that lineage. Contour lines marked 0.025 define areas in which there is a high probability of cases being caused by a given lineage, suggesting spatial segregation. Contour lines marked 0.975 define areas in which there is a low probability of cases being caused by the given lineage. From: Tarr, G. A. M., et al. (2018). "Geogenomic Segregation and Temporal Trends of Human Pathogenic *Escherichia coli* O157:H7, Washington, USA, 2005–2014." *Emerging Infectious Diseases* 24(1): 32-39. Article is from the journal *Emerging and Infectious Diseases*, which is in the public domain.**

#### 7.4.4. Conclusion.

Bauer et al. (2015) state that “the quiet revolution of numerical weather prediction has required combined scientific, observing, and computational technology advances to be made” and can be “the solution to large problems, such as simulating the neurological connectivity of the human brain or the evolution of the galaxies in the cosmos.”<sup>417</sup> If such possibilities exist, surely policymakers can apply the same verbosity to solving food safety issues and identifying future problems. For example, the accumulation of STEC and *Salmonella* outbreak data (see chapter 5) can be leveraged to try and assess where a new outbreak can occur. Or, this data can

<sup>417</sup> Bauer, Thorpe, and Brunet., p. 53.

be combined with environmental, physical, chemical, economic, and supply-chain metrics (available through the public-private partnerships advocated for in chapter 6) to pinpoint vulnerability areas within the system that may require more control to reduce food safety risk.

## **7.5. STRATEGY 5: ADVOCATE MULTIDISCIPLINARY TEACHING.**

### **7.5.1. The (ultimate?) long-term strategy: advocate multidisciplinary thinking.**

The connection between performing multidisciplinary thinking and the food system dates back to the beginning of time:

*The advent of cooking enabled humans to eat more kinds of food, to devote less time to eating, and to make do with smaller teeth and shorter intestines. Some scholars believe there is a direct link between the advent of cooking, the shortening of the human intestinal track, and the growth of the human brain...Whereas chimpanzees spend five hours a day chewing raw food, a single hour suffices for people eating cooked food.*<sup>418</sup>

Scientific advances “require more interdisciplinary research at the science-technology interface.”<sup>419</sup> Being multidisciplinary makes one more curious as well as aware. As Walter Isaacson, a biographer who has chronicled the lives of Benjamin Franklin, Albert Einstein, Steve Jobs, and Leonardo da Vinci, finds multidisciplinary thinking the most attractive characteristic of his subjects, stating that “the ability to make connections across disciplines—arts and sciences, humanities and technology—is a key to innovation, imagination, and genius.”<sup>420</sup>

Leonardo da Vinci is, of course, the original “Renaissance Man.” Or, put another way, da Vinci is the preeminent multidisciplinary thinker, blending lessons from art and design to inform science and engineering, and vice versa. The genius had an “ability to combine art and science,”

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<sup>418</sup> Yuval Noah Harari, “An Animal of No Significance,” in *Sapiens* (New York, NY: HarperCollins, 2015)., p. 12-13.

<sup>419</sup> Bauer, Thorpe, and Brunet., p. 53.

<sup>420</sup> Walter Isaacson, “I Can Also Paint,” in *Leonardo da Vinci* (New York, NY: Simon & Schuster, 2017)., pp. 2-3.

and, “with a passion that was both playful and obsessive, he pursued innovative studies of anatomy, fossils, birds, the heart, flying machines, optics, botany, geology, water flows and weaponry.”<sup>421</sup> If the future food safety crises are going to be identified and solved, programs should *nurture da Vinci*’s: encouraging today’s food science students to be “wildly imaginative, passionately curious, and creative across multiple disciplines.”<sup>422</sup>

For an American example of the benefits of the multidisciplinary life, one need look no further than the author of the Declaration of Independence and the third President of the United States, Thomas Jefferson. The Sage of Monticello was “a philosopher and a scientist, a naturalist and a historian...always looking forward, consumed by the quest for knowledge.”<sup>423</sup> Jefferson, who would, in addition to the Declaration and Presidency, author the Virginia Statute for Religious Freedom and found the University of Virginia, was known for his attention-to-detail, inventiveness, and inquisitive nature.<sup>424</sup> While Jefferson will be remembered for what he did politically, the impact of his diverse, multidisciplinary approach to life is evident. As his biographer Jon Meacham states:

*Had he been only a philosopher he would not have endured as he does. Had he been only a legislator, or only a diplomat, or only an inventor, or only an author, or only an educator, or even only a president he would not have endured as he does...He endures because we can see in him all the varied and wondrous possibilities of the human experience—the thirst for knowledge, the capacity to create, the love of family and of friends, the hunger for accomplishment, the applause of the world, the marshaling of power, the bending of others to one’s vision. His genius lay in his versatility; his larger political legacy in this leadership of thought and of men.*<sup>425</sup>

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<sup>421</sup> Ibid., p. 1.

<sup>422</sup> Ibid., p. 3.

<sup>423</sup> Jon Meacham, "Prologue: The World's Best Hope," in *Thomas Jefferson: The Art of Power* (New York, NY: Random House Trade Paperbacks, 2012), p. xxi. The author’s interest in Jefferson began when visiting his home, Monticello, while on a *Frontier* field trip in 2015.

<sup>424</sup> Ibid. Jefferson is said to have dabbled in archaeology, paleontology, astronomy, botany, meteorology, interior design, accounting, music, gardening, and Biblical studies.

<sup>425</sup> "Epilogue: All Honor to Jefferson," p. 499.

Another example of multidisciplinary thinking “paying off” is the work of polymath and public health pioneer John Snow, who figured out the cholera waterborne mystery in 1854-55. He and his colleague, Henry Whitehead, took a local’s knowledge of the SoHo community and viewed it from the bird’s eye perspective: this would become “a model for managing and sharing information that has implications that extend far beyond epidemiology.”<sup>426</sup> Snow’s work on identifying the source of the cholera outbreak was dependent on two key principles. One was the importance of amateurs and novices. More pertinent to this strategy’s discussion is the second principle: “the lateral, cross-disciplinary flow of ideas.”<sup>427</sup> Snow met people in the coffeehouses of London where interests and skills overlap, where people from different industries and walks of life share anecdotes and opinions. As Johnson (2006) states:

*Snow himself was a kind of one-man coffeehouse: One of the primary reasons he was able to cut through the fog of miasma was his multidisciplinary approach, as a practicing physician, mapmaker, inventor, chemist, demographer, and medical detective. But even with that polymath background, he still needed to draw upon an entirely different set of skills.*<sup>428</sup>

Taking the lessons from these tremendous thought-leaders, it is clear why advocating for multidisciplinary thinking should be the goal of policymakers looking to identify the next unknown unknown. The big issues are going to be more quickly identified and solved by minds that are flexible, curious, and able to cross disciplines. This has been echoed in policy: the National Academy of Sciences, in a major 2005 report, advocate for interdisciplinary (multidisciplinary thinking) as a means to address the complexity of nature and society, to solve

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<sup>426</sup> Johnson., p. 225.

<sup>427</sup> Ibid. p. 225.

<sup>428</sup> Ibid., p. 226.

major problems, and to explore new technologies.<sup>429</sup> As another example, in the past decade DHS has awarded numerous career development grants (CDG) for students to participate in interdisciplinary studies related to homeland security. Finally, the *Frontier* program at Kansas State University, co-directed by Dr. Justin J. Kastner and Dr. Jason M. Ackleson, has trained multidisciplinary students eager to become scholarly, thoughtful leaders in the areas of food safety and security, cross-border cooperation, and international trade policy. Over 300 students have attended *Frontier* field trips, where students experience the complexity of global issues by real-world visits to international trade ports-of-entry, food manufacturing plants and distribution facilities, libraries, museums, and governmental and non-governmental policy-making and policy-analysis groups. These trips to various cities, including Washington, D.C., New Orleans, Los Angeles, and Boston, alongside interactions with public- and private-sector officials, have helped students acquire multidisciplinary insights across the multiple facets of the global food system. The next da Vinci, Jefferson, or Snow of the food industry may very well have had his or her life goals crystallized on a *Frontier* field trip.

Thus, a well-rounded, multidisciplinary education policy is advocated. Indeed, education policymakers should encourage at all levels curricula that bridge the different disciplines. Such an endeavor will propel those same students into college, university, or careers where the multidisciplinary thinking can continue, and, as hypothesized here, assist in solving the big problems facing society.

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<sup>429</sup> National Academy of Sciences, "Facilitating Interdisciplinary Research," (Washington, D.C.: National Academy of Sciences, National Academy of Engineering, and Institute of Medicine, 2005). One method for a graduate student, according to the report, is to write a dissertation that involves multiple advisors in different disciplines (p. 193). Such is the case in this dissertation.

## 7.6. CONCLUSION

Five strategies have been proposed to address unknown unknowns, particularly those facing the food industry; however, the endeavor of identifying unknown unknowns, and in turn, moving them to known unknowns is a worthwhile exercise for any discipline. The first strategy, learning from the past, was demonstrated by a watershed unknown unknown event in the food system: the German O104 outbreak. Microbiologists and public health officials should study the German outbreak to better anticipate the next time such an event occurs, which *will* happen. The second and third strategies are immediate actions that can be taken. Strategy two advocates understanding products of increasing or high consumption by the general public: by knowing unusual or novel eating trends, higher-risk foods, as well as foods that are produced in a way that may promote a food safety event, can be identified. The third strategy encourages interdependent and cross-cutting critical infrastructures to be aware of each other's vulnerabilities, as often one vulnerability will overlap among critical infrastructures. The interdependence of IT and FA is a relevant example. The fourth strategy is to borrow strategies from meteorology. While envisaging the next food safety crisis is not like forecasting the weather, the *principles* embraced by the meteorological community serve as pertinent ideals for food safety professionals. Finally, the benefit of multidisciplinary thinking is discussed, and, through the stories of influential multidisciplinary thinkers, an educational policy is proposed.

The future of food safety is very much a world of the unknown. That unknown may result in positive advances in science, or it may result in challenges that must be confronted. To conclude this chapter, several questions (or unknown unknowns) are posed. These questions serve not only as educated guesses about the future of food safety, but also act as areas for future research which are encouraged:

1. As microbiological technique improves, will we have the capability to detect and identify new genera of foodborne pathogens?
2. What impact will novel, evolving technologies, such as clustered regularly interspaced short palindromic repeats (CRISPRs), have on identification and mitigation of pathogens like STEC? How will the loss of spacers and changing of loci during modular assortment change those capabilities?<sup>430</sup>
3. Will increasing international travel and trade spread virulent pathogens (*e.g.*, *E. coli* O104:H4, as discussed in this dissertation) from other parts of the world to the U.S.? What can be done to slow the spread of, or at least more quickly identify, non-innate pathogens?
4. What effect will climate change have on food safety?
  - a. Cattle shed more STEC during warmer months (“super shedders”).<sup>431</sup> Will rising temperatures and/or longer summers impact STEC shedding?
  - b. Rising sea temperatures have led to an emerging *Vibrio* spp. risk at high latitudes.<sup>432</sup> Will rising sea temperatures result in more foodborne *Vibrio* infections, and how will that impact the seafood industry?
  - c. Data demonstrates that rising temperatures, short-term temperature changes, and changes in precipitation often result in increasing cases of

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<sup>430</sup> Sabine Delannoy, Lothar Beutin, and Patrick Fach, "Improved Traceability of Shiga-Toxin-Producing *Escherichia coli* Using CRISPRs for Detection and Typing," *Environmental Science and Pollution Research* 23, no. 9 (2016).

<sup>431</sup> Tom S. Edrington et al., "Seasonal Shedding of *Escherichia coli* O157:H7 in Ruminants: A New Hypothesis," *Foodborne Pathogens and Disease* 3, no. 4 (2006).

<sup>432</sup> Craig Baker-Austin et al., "Emerging *Vibrio* Risk at High Latitudes in Response to Ocean Warming," *Nature Climate Change* 3 (2012).

salmonellosis, campylobacteriosis, and foodborne trematode infections.<sup>433</sup>

How can this information be used to inform public health officials?

5. How would the government (particularly FDA or USDA) respond to a highly-virulent foodborne pathogen, introduced intentionally or unintentionally? For example, can responses to the Ebola outbreak in West Africa or the Zika virus in South America shed light on what might happen if a panic-inducing pathogen was found in the food system? What roles would the government and the private sector play?
6. How will automation and artificial intelligence impact the food system and food safety? For example, Japan plans to launch self-navigating cargo ships by 2025.<sup>434</sup>
7. How will the changing population impact food safety? The population of retired and elderly is increasing, as is the population of persons immunosuppressed by disease or treatment.<sup>435</sup>
8. What good news will scientists discover? Evidence has shown that bacteriocins, which are antimicrobial peptides produced by certain bacteria, may serve as effective alternatives to antibiotics.<sup>436</sup> Teixobactin, a soil-derived antibiotic, kills bacteria without detectable resistance development.<sup>437</sup> Some unknown unknowns will be serendipitous and inevitably beneficial to humanity.

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<sup>433</sup> M. C. Tirado et al., "Climate Change and Food Safety: A Review," *Food Research International* 43, no. 7 (2010).

<sup>434</sup> BBC, "Japan to Launch Self-Navigating Cargo Ships 'by 2025'," <http://www.bbc.com/news/technology-40219682>.

<sup>435</sup> R. V. Tauxe et al., "Evolving Public Health Approaches to the Global Challenge of Foodborne Infections," *International Journal of Food Microbiology* 139 (2010).

<sup>436</sup> Paul D. Cotter, R. Paul Ross, and Colin Hill, "Bacteriocins — a Viable Alternative to Antibiotics?," *Nature Reviews Microbiology* 11 (2012).

<sup>437</sup> Losee L. Ling et al., "A New Antibiotic Kills Pathogens without Detectable Resistance," *Nature* 517, no. 7535 (2015).

These questions, and vast numbers more, will dictate food safety in the U.S. and the globe in the future. It is the hope of the author that food system policymakers will embrace the five strategies advocated in this chapter so that solving inevitable problems becomes simpler and quicker for the benefit of the global citizenry.

## Postscript

In the conclusion of his biography on da Vinci, William Isaacson offers several “learnings from Leonardo.”<sup>438</sup> Striving to be a multidisciplinary thinker like da Vinci is very much a worthwhile pursuit, one which this author has embraced during his doctoral program of study. While Isaacson identified twenty learnings from Leonardo, at least seven of these were confirmed during this doctoral program and (hopefully) demonstrated in this dissertation: (1) observe, (2) start with the details, (3) see things unseen, (4) go down rabbit holes, (5) avoid silos, (6) collaborate, and (7) be open to mystery.<sup>439</sup>

This dissertation began with a series of *observations* that were formed through experimentally obtained *details*. The interaction of stress and antimicrobials was examined, and found to be small. These details, while not guaranteeing that bacterial cross-protection or stress adaptation to sanitizers is a certainty, gives regulators confidence that current prescribed antimicrobial concentrations will continue to control STEC and *Salmonella*. Experimentation on the attachment of STEC to lean and adipose beef tissue was an amalgamation of time points, temperatures, and growth conditions (*facts* and *observations*). These demonstrated that, while fat content does not necessarily determine STEC attachment, time, temperature, and metabolic state of the bacteria (particularly those entering the abattoir on the hide of an animal) may impact antimicrobial efficacy. Finally, work with a combined chemical intervention (SBS-PAA) on spinach demonstrated that such an approach may be more effective than the use of chlorine.

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<sup>438</sup> Isaacson, "Conclusion.", pp. 519-524.

<sup>439</sup> Ibid.

The author is grateful to his advisors that he was able to *go down the rabbit hole* and find a food safety and policy topic that was compelling and interesting: unknown unknowns. In doing so, this dissertation produced valuable analysis on the resilience of the food system and its ability to withstand a food-related catastrophe. Leaping head-first into such an endeavor resulted in a series of strategies that food regulators and policymakers at-large can use to better support the food system and sustain its public- and private-sector stakeholders and stewards. Part Two, the policy component of this dissertation, was an exercise in *avoiding silos*: much like “Leonardo had a free-range mind that merrily wandered across all the disciplines of the arts, science, engineering, and humanities,”<sup>440</sup> Part Two of this dissertation blends disciplinary insights from microbiology, political science, history, and even meteorology; this free-ranging exploration allowed the author to wander not into unimportant trivia but key food-system topics like resilience and regulation. *Collaboration* is the action of working with others to produce or create something. The multitude of persons that have impacted this dissertation have been accounted for in the acknowledgements, and their contributions are warmly appreciated. This dissertation is also a collaboration of ideas and concepts that support and reinforce one another: Part Two’s policy frameworks and strategies beg new questions related to Part One’s laboratory-based questions, and Part One’s conclusions inform what kinds of policy prescriptions are most realistic.

Leonardo da Vinci’s final learning is to be open to mystery. While some mysteries have been unveiled in this dissertation, it is the unanswered mysteries that will continue to enchant and inspire food microbiologists and food policymakers well into the future. Albert Einstein once said:

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<sup>440</sup> Ibid., p. 522.

*The important thing is not to stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery each day. Never lose a holy curiosity.*

The author is grateful to his mentors for being encouraged to enjoy both mystery and curiosity.

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## Appendix A - Microplate Growth, Intermediate Growth, and Susceptibility Results by Rep for STEC and *Salmonella*

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 20 ppm |   |   | 25 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | G | G | G      | G | G | G      | G | G | G      | S | G | G      | S | G | G      | S | G |
| ACID    | G     | G | G | G      | G | G | G      | G | G | S      | S | G | S      | S | G | S      | S | G |
| ALK     | G     | G | G | G      | G | G | G      | G | G | I      | S | G | G      | S | G | S      | S | G |
| FT      | G     | G | G | G      | G | G | G      | G | G | G      | S | G | G      | S | G | S      | S | G |
| HEAT    | G     | G | G | G      | I | G | G      | G | G | I      | S | S | G      | S | S | S      | S | S |
| SALT    | G     | G | G | G      | G | G | G      | G | G | I      | S | G | G      | S | G | S      | S | G |

(a)

|         | 1.50% |   |   | 1.75% |   |   | 2% |   |   | 2.25% |   |   | 2.50% |   |   | 2.75% |   |   |
|---------|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | I  | G | G | S     | I | S | S     | G | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | I  | G | I | S     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | I  | G | I | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | G  | G | G | I     | I | I | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | I | I | S  | I | S | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | I  | G | I | S     | I | S | S     | S | S | S     | S | S |

(b)

|         | 80 ppm |   |   | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   |
|---------|--------|---|---|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | G      | G | G | G      | G | G | G       | G | G | I       | G | S | S       | I | S | S       | S | S |
| ACID    | G      | G | G | G      | G | G | G       | G | S | S       | I | S | S       | S | S | S       | S | S |
| ALK     | G      | G | G | G      | G | G | G       | G | G | S       | G | S | S       | I | S | S       | I | I |
| FT      | G      | G | G | G      | G | G | G       | G | S | I       | G | S | S       | G | S | S       | S | S |
| HEAT    | G      | I | I | I      | S | I | S       | S | I | S       | S | I | S       | S | I | S       | S | S |
| SALT    | G      | G | G | G      | G | G | G       | G | G | G       | G | G | S       | G | G | S       | I | S |

(c)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | I | G     | G | I | I     | I | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | I | G     | I | I | I     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | I | G | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | I | G     | G | I | I     | I | S | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | G | S | I     | S | S | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | I | I     | G | I | I     | S | S | S     | S | S | S     | S | S |

(d)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | I | I     | I | S | S     | S | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | I     | S | I | S     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | S     | S | G | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | I     | I | S | S     | S | S | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | I | S | S     | S | S | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | S     | S | I | S     | S | S | S     | S | S | S     | S | S |

(e)

**Figure A.1: Impact of stress on *Salmonella* Montevideo.**

Growth (G), intermediate growth (I), or susceptibility (S) of *Salmonella* Montevideo by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 450 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 20 ppm |   |   | 25 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | G | G | G      | G | G | G      | G | G | S      | G | G | S      | I | G | S      | I | S |
| ACID    | G     | G | G | G      | G | G | G      | G | G | S      | G | G | S      | I | G | S      | I | S |
| ALK     | G     | G | G | G      | G | G | G      | G | G | S      | G | G | S      | G | G | S      | G | S |
| FT      | G     | G | G | G      | G | G | G      | G | G | S      | G | G | S      | I | G | S      | I | S |
| HEAT    | G     | G | G | S      | G | G | G      | G | G | S      | G | G | S      | S | G | S      | S | S |
| SALT    | G     | G | G | G      | G | G | G      | G | G | S      | G | G | S      | G | G | S      | G | G |

(a)

|         | 1.50% |   |   | 1.75% |   |   | 2% |   |   | 2.25% |   |   | 2.50% |   |   | 2.75% |   |   |
|---------|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G  | G | G | I     | S | I | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | G  | G | G | S     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G  | G | G | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | G  | I | G | I     | S | I | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | S     | S | G | S  | S | I | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G  | G | G | S     | S | I | S     | S | S | S     | S | S |

(b)

|         | 80 ppm |   |   | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   |
|---------|--------|---|---|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | G      | G | G | G      | G | G | G       | G | G | I       | S | I | S       | S | S | S       | S | S |
| ACID    | G      | G | G | G      | G | G | G       | S | G | S       | I | G | S       | S | S | S       | S | S |
| ALK     | G      | G | G | G      | G | G | G       | S | G | S       | S | I | S       | S | S | S       | S | S |
| FT      | G      | G | G | G      | G | G | G       | S | G | S       | S | S | S       | S | S | S       | S | S |
| HEAT    | S      | S | G | S      | S | G | S       | S | S | S       | S | S | S       | S | S | S       | S | S |
| SALT    | G      | G | G | G      | G | G | G       | G | G | G       | G | G | G       | G | G | S       | S | S |

(c)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G     | G | G | I     | G | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | G     | G | G | I     | S | G | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | G | G | I     | I | I | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | I | G | G     | G | I | G     | I | G | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | S | G | S     | S | I | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G     | G | G | I     | G | S | I     | S | S | S     | S | S |

(d)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | I     | I | G | S     | S | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | S     | S | G | S     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | S     | I | I | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | I     | S | I | S     | S | S | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | S     | S | G | S     | S | S | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | S     | G | G | S     | S | S | S     | S | S | S     | S | S |

(e)

**Figure A.2: Impact of stress on *Salmonella* Newport.**

Growth (G), intermediate growth (I), or susceptibility (S) of *Salmonella* Newport by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 450 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 20 ppm |   |   | 25 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | G | G | G      | G | G | G      | G | G | S      | G | S | S      | I | G | S      | S | S |
| ACID    | G     | G | G | G      | G | G | G      | G | G | S      | G | S | S      | G | G | S      | S | S |
| ALK     | G     | G | G | G      | G | G | G      | G | G | S      | G | S | S      | G | G | S      | I | S |
| FT      | G     | G | G | G      | G | G | G      | G | G | S      | G | S | S      | G | S | S      | S | I |
| HEAT    | G     | G | G | S      | G | G | G      | G | G | S      | G | S | S      | S | G | S      | S | S |
| SALT    | G     | G | G | S      | G | G | G      | G | G | S      | G | S | S      | I | G | S      | I | S |

(a)

|         | 1.50% |   |   | 1.75% |   |   | 2% |   |   | 2.25% |   |   | 2.50% |   |   | 2.75% |   |   |
|---------|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G  | I | G | I     | S | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | I | G | G  | I | G | S     | S | S | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G  | G | I | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | G  | G | G | I     | S | S | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | I     | I | G | S  | S | I | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G  | I | G | I     | S | I | S     | S | S | S     | S | S |

(b)

|         | 80 ppm |   |   | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   |
|---------|--------|---|---|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | G      | G | G | G      | G | G | G       | G | G | S       | S | G | S       | S | S | S       | S | S |
| ACID    | G      | I | G | G      | G | G | G       | S | G | S       | S | S | S       | S | S | S       | S | S |
| ALK     | G      | G | G | G      | G | G | G       | S | G | S       | S | S | S       | S | S | S       | S | S |
| FT      | G      | G | G | G      | G | G | G       | S | G | S       | S | S | S       | S | S | S       | S | S |
| HEAT    | S      | S | G | S      | S | S | S       | S | S | S       | S | S | S       | S | S | S       | S | S |
| SALT    | G      | G | G | G      | G | G | G       | G | G | G       | G | S | G       | G | S | S       | S | S |

(c)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G     | G | G | I     | S | I | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | G     | G | G | I     | S | I | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | G | G | I     | S | I | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | I | G | G     | I | G | I     | S | I | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | S | G | I     | I | I | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G     | G | G | S     | I | I | S     | S | S | S     | S | S |

(d)

|         | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   | 0.90% |   |   | 1.00% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | S     | I | I | S     | S | S | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | G | G | S     | S | I | S     | S | S | I     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | S     | S | I | S     | S | S | S     | S | S | S     | S | S |
| FT      | G     | G | G | G     | G | G | S     | I | I | S     | S | S | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | S | G | S     | S | S | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | S     | I | G | S     | S | S | S     | S | S | S     | S | S |

(e)

**Figure A.3: Impact of stress on *Salmonella* Typhimurium.**

Growth (G), intermediate growth (I), or susceptibility (S) of *Salmonella* Montivideo by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 450 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 17.5 ppm |   |   | 20 ppm |   |   | 22.5 ppm |   |   | 25 ppm |   |   | 27.5 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | - | - | G      | - | - | G      | - | - | -        | S | G | G      | S | I | -        | S | S | S      | S | S | -        | S | S | S      | S | S |
| ACID    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | I | S      | I | S | -        | S | S | S      | S | S |
| ALK     | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | I | S      | I | S | -        | S | S | S      | S | S |
| FT      | G     | - | - | G      | - | - | G      | - | - | -        | G | I | G      | G | I | -        | G | S | S      | G | S | -        | S | S | S      | S | S |
| HEAT    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | S | -        | G | S | S      | G | S | -        | S | S | S      | S | S |
| SALT    | G     | - | - | G      | - | - | G      | - | - | -        | G | S | G      | G | S | -        | G | S | S      | S | S | -        | S | S | S      | S | S |

(a)

|         | 1.00% |   |   | 1.25% |   |   | 1.50% |   |   | 1.75% |   |   | 2.00% |   |   | 2.25% |   |   | 2.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | G | G | G     | G | G | I     | S | G | S     | S | S | S     | S | I | -     | S | S |
| ACID    | G     | - | - | G     | G | G | I     | I | G | S     | S | G | S     | S | G | S     | S | G | -     | S | S |
| ALK     | G     | - | - | G     | G | G | G     | S | G | S     | S | G | S     | S | G | S     | S | G | -     | S | I |
| FT      | G     | - | - | G     | G | G | G     | S | G | S     | S | G | S     | S | I | S     | S | I | -     | S | I |
| HEAT    | G     | - | - | G     | G | S | I     | I | S | S     | S | S | S     | S | S | S     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | S | G | I     | S | G | S     | S | I | S     | S | S | S     | S | S | -     | S | S |

(b)

|         | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   | 140 ppm |   |   | 150 ppm |   |   | 160 ppm |   |   |
|---------|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | -      | S | G | -       | S | G | I       | S | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| ACID    | -      | S | G | -       | G | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| ALK     | -      | S | G | -       | S | G | I       | S | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| FT      | -      | S | G | -       | S | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| HEAT    | -      | S | G | -       | S | G | S       | S | S | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| SALT    | -      | S | G | -       | S | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |

(c)

|         | 0.25% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.75% |   |   | 0.80% |   |   | 0.90% |   |   | 1% |   |   | 1.25% |   |   | 1.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | -     | G | I | G     | I | G | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| ACID    | -     | G | G | G     | I | I | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| ALK     | -     | I | G | G     | G | G | S     | - | - | S     | - | - | -     | S | G | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| FT      | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | S | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| HEAT    | -     | G | G | G     | S | I | S     | - | - | S     | - | - | -     | S | S | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| SALT    | -     | G | G | G     | I | G | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |

(d)

|         | 0.30% |   |   | 0.40% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G     | S | I | I     | S | I | S     | S | S | S     | S | S |
| ACID    | G     | G | G | G     | I | G | I     | S | G | S     | S | G | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | S | G | S     | S | G | S     | S | I | S     | S | S |
| FT      | G     | G | G | G     | G | G | G     | S | G | S     | S | I | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | G | G | G     | S | G | S     | S | I | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G     | S | G | S     | S | I | S     | S | S | S     | S | S |

(e)

**Figure A.4: Impact of stress on Shiga toxin-producing *Escherichia coli* (STEC) O26**

Growth (G), intermediate growth (I), or susceptibility (S) of STEC O26 by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 595 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 17.5 ppm |   |   | 20 ppm |   |   | 22.5 ppm |   |   | 25 ppm |   |   | 27.5 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | G | G | -        | S | G | S      | S | S |
| ACID    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | G      | S | G | -        | S | G | G      | S | S |
| ALK     | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | G | I | -        | S | S | S      | S | S |
| FT      | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | G | S | -        | S | S | S      | S | S |
| HEAT    | G     | - | - | G      | - | - | G      | - | - | -        | G | S | G      | G | S | -        | G | S | S      | G | S | -        | I | S | S      | S | S |
| SALT    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | G | S | -        | S | S | S      | S | S |

(a)

|         | 1.00% |   |   | 1.25% |   |   | 1.50% |   |   | 1.75% |   |   | 2.00% |   |   | 2.25% |   |   | 2.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | G | G | G     | I | G | S     | S | G | S     | S | G | S     | S | G | -     | S | G |
| ACID    | G     | - | - | G     | G | G | G     | S | G | S     | G | I | S     | G | G | S     | G | - | S     | S |   |
| ALK     | G     | - | - | G     | G | G | G     | G | G | S     | G | S | S     | G | S | S     | G | - | S     | S |   |
| FT      | G     | - | - | G     | G | G | G     | G | G | S     | S | G | S     | S | G | S     | S | G | -     | S | I |
| HEAT    | G     | - | - | G     | G | S | G     | G | S | S     | S | S | S     | S | S | S     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | G | G | G     | G | G | S     | S | G | S     | S | G | S     | S | G | -     | S | G |

(b)

|         | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   | 140 ppm |   |   | 150 ppm |   |   | 160 ppm |   |   |
|---------|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | -      | G | G | -       | G | G | S       | S | G | S       | S | I | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| ACID    | -      | G | G | -       | G | G | G       | S | G | G       | S | S | G       | S | S | G       | S | S | G       | - | - | G       | - | - |
| ALK     | -      | G | G | -       | G | G | G       | G | I | G       | S | S | G       | S | S | S       | S | S | S       | - | - | S       | - | - |
| FT      | -      | G | G | -       | I | G | S       | S | G | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| HEAT    | -      | G | G | -       | S | I | S       | I | S | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| SALT    | -      | G | G | -       | G | G | G       | G | G | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |

(c)

|         | 0.25% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.75% |   |   | 0.80% |   |   | 0.90% |   |   | 1% |   |   | 1.25% |   |   | 1.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 |       |   |   |
| CONTROL | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | G | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| ACID    | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| ALK     | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | G | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| FT      | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| HEAT    | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | S | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |
| SALT    | -     | G | G | G     | G | G | S     | - | - | S     | - | - | -     | S | I | S     | - | - | S     | - | - | S  | S | S | -     | S | S | -     | S | S |

(d)

|         | 0.30% |   |   | 0.40% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G     | G | G | S     | S | G | S     | S | I | S     | S | S |
| ACID    | G     | G | G | G     | G | G | G     | I | G | I     | S | G | I     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | G | G | G     | S | G | S     | S | I | S     | S | S |
| FT      | G     | G | G | G     | G | G | G     | G | G | S     | S | G | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | G | G | G     | G | G | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | G     | G | G | G     | G | G | S     | S | G | S     | S | S | S     | S | S |

(e)

**Figure A.5: Impact of stress on Shiga toxin-producing *Escherichia coli* (STEC) O45**

Growth (G), intermediate growth (I), or susceptibility (S) of STEC O45 by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 595 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 17.5 ppm |   |   | 20 ppm |   |   | 22.5 ppm |   |   | 25 ppm |   |   | 27.5 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | S | G | S      | S | G | -        | S | I | S      | S | S |
| ACID    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | S | I | S      | S | G | -        | S | S | S      | S | S |
| ALK     | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | S | G | S      | S | I | -        | I | S | S      | S | S |
| FT      | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | I | G | -        | S | G | S      | S | S |
| HEAT    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | S | S | -        | S | S | S      | S | S |
| SALT    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | I | G | S      | S | I | -        | S | I | S      | S | S |

(a)

|         | 1.00% |   |   | 1.25% |   |   | 1.50% |   |   | 1.75% |   |   | 2.00% |   |   | 2.25% |   |   | 2.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | I     | G | G | I     | I | G | S     | S | G | S     | S | G | S     | S | G | -     | S | G |
| ACID    | G     | - | - | G     | I | G | G     | G | G | I     | S | G | S     | S | G | S     | S | G | -     | S | G |
| ALK     | G     | - | - | G     | I | G | G     | I | G | I     | S | G | S     | S | G | S     | S | G | -     | S | G |
| FT      | I     | - | - | G     | G | G | I     | G | G | S     | S | G | S     | S | G | S     | S | G | -     | S | G |
| HEAT    | G     | - | - | G     | I | S | I     | I | S | S     | S | S | S     | S | S | S     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | G | G | I     | G | G | S     | S | G | S     | S | G | S     | S | G | -     | S | I |

(b)

|         | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   | 140 ppm |   |   | 150 ppm |   |   | 160 ppm |   |   |
|---------|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | -      | G | G | -       | G | G | I       | I | I | S       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| ACID    | -      | G | G | -       | G | G | G       | I | G | G       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| ALK     | -      | G | G | -       | S | G | I       | I | G | S       | S | G | S       | S | S | S       | S | S | S       | - | - | S       | - | - |
| FT      | -      | G | G | -       | G | G | S       | G | I | S       | I | S | S       | S | S | S       | S | I | S       | - | - | S       | - | - |
| HEAT    | -      | I | G | -       | S | G | S       | S | I | S       | S | S | S       | S | S | S       | S | I | S       | - | - | S       | - | - |
| SALT    | -      | G | G | -       | I | G | I       | I | G | I       | S | S | S       | S | S | S       | S | S | S       | - | - | S       | - | - |

(c)

|         | 0.25% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.75% |   |   | 0.80% |   |   | 0.90% |   |   | 1% |   |   | 1.25% |   |   | 1.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|----|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1  | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | -     | G | G | G     | G | G | S     | - | - | S     | - | - | S     | S | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |
| ACID    | -     | G | G | G     | G | G | S     | - | - | S     | - | - | S     | G | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |
| ALK     | -     | G | G | G     | G | G | S     | - | - | S     | - | - | S     | I | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |
| FT      | -     | G | G | G     | G | G | S     | - | - | S     | - | - | S     | I | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |
| HEAT    | -     | G | G | I     | I | G | S     | - | - | S     | - | - | S     | S | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |
| SALT    | -     | G | G | G     | G | G | S     | - | - | S     | - | - | S     | I | S | -     | - | S | -     | - | S | S  | S | - | S     | S | - | S     | S |   |

(d)

|         | 0.30% |   |   | 0.40% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | G | G | G     | G | G | G     | I | G | S     | S | G | S     | S | I | S     | S | S |
| ACID    | G     | G | G | G     | G | G | G     | G | G | I     | S | G | S     | S | S | S     | S | S |
| ALK     | G     | G | G | G     | G | G | G     | G | G | I     | G | G | S     | S | I | S     | S | S |
| FT      | G     | G | G | I     | G | G | S     | G | G | S     | S | G | S     | S | S | S     | S | S |
| HEAT    | G     | G | G | G     | G | G | G     | I | G | S     | S | S | S     | S | S | S     | S | S |
| SALT    | G     | G | G | I     | G | G | S     | G | G | S     | I | G | S     | S | S | S     | S | S |

(e)

**Figure A.6: Impact of stress on Shiga toxin-producing *Escherichia coli* (STEC) O103**

Growth (G), intermediate growth (I), or susceptibility (S) of STEC O103 by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 595 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 17.5 ppm |   |   | 20 ppm |   |   | 22.5 ppm |   |   | 25 ppm |   |   | 27.5 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | I | G | -        | I | G | I      | S | S | -        | S | S | S      | S | S |
| ACID    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | I | G | I      | S | I | -        | S | G | I      | S | S |
| ALK     | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | I | S      | S | S | -        | I | S | S      | S | S |
| FT      | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | G | G | S      | G | S | -        | I | S | S      | S | S |
| HEAT    | G     | - | - | G      | - | - | G      | - | - | -        | I | G | G      | S | G | -        | S | G | S      | S | I | -        | S | S | S      | S | S |
| SALT    | G     | - | - | G      | - | - | I      | - | - | -        | G | I | G      | G | I | -        | S | I | S      | S | S | -        | S | S | S      | S | S |

(a)

|         | 1.00% |   |   | 1.25% |   |   | 1.50% |   |   | 1.75% |   |   | 2.00% |   |   | 2.25% |   |   | 2.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | G | G | G     | S | I | G     | S | I | S     | S | S | S     | S | S | -     | S | S |
| ACID    | G     | - | - | G     | G | G | G     | S | G | G     | S | S | G     | S | S | G     | S | S | -     | S | S |
| ALK     | G     | - | - | G     | G | G | G     | I | G | G     | S | G | I     | S | S | S     | S | S | -     | S | S |
| FT      | G     | - | - | G     | G | G | G     | I | G | G     | I | G | S     | S | S | S     | S | S | -     | S | S |
| HEAT    | G     | - | - | G     | S | I | G     | S | G | G     | S | S | S     | S | I | S     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | G | G | G     | S | G | G     | S | G | S     | S | S | S     | S | S | -     | S | S |

(b)

|         | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   | 140 ppm |   |   |
|---------|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | G      | G | G | S       | S | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S |
| ACID    | G      | G | G | I       | S | G | I       | S | G | I       | S | S | I       | S | S | I       | S | S |
| ALK     | G      | I | G | I       | I | G | I       | S | I | I       | S | S | I       | S | S | S       | S | S |
| FT      | G      | G | G | S       | G | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S |
| HEAT    | S      | S | G | S       | S | G | S       | S | S | S       | S | S | S       | S | S | S       | S | S |
| SALT    | S      | I | G | G       | I | G | S       | S | S | S       | S | S | S       | S | S | S       | S | S |

(c)

|         | 0.125% |   |   | 0.250% |   |   | 0.500% |   |   | 0.750% |   |   | 1.000% |   |   | 1.250% |   |   |
|---------|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|
| REP →   | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G      | - | - | G      | G | G | G      | I | G | S      | S | S | S      | S | S | S      | S | S |
| ACID    | G      | - | - | G      | G | G | G      | G | G | S      | S | S | S      | S | S | S      | S | S |
| ALK     | G      | - | - | G      | G | G | G      | G | G | S      | S | S | S      | S | S | S      | S | S |
| FT      | G      | - | - | G      | G | G | I      | G | G | S      | S | S | S      | S | S | S      | S | S |
| HEAT    | G      | - | - | G      | G | G | I      | S | G | S      | S | S | S      | S | S | S      | S | S |
| SALT    | G      | - | - | G      | G | G | G      | G | G | S      | S | S | S      | S | S | S      | S | S |

(d)

|         | 0.05% |   |   | 0.10% |   |   | 0.20% |   |   | 0.30% |   |   | 0.40% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | I | G | I     | S | G | -     | S | I | -     | S | S | -     | S | S |
| ACID    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | I     | S | G | -     | S | I | -     | S | S | -     | S | S |
| ALK     | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | I     | S | G | -     | S | I | -     | S | S | -     | S | S |
| FT      | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | S     | G | G | -     | I | I | -     | S | S | -     | S | S |
| HEAT    | G     | - | - | G     | - | - | G     | - | - | G     | I | G | G     | S | G | S     | S | G | -     | S | S | -     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | I     | I | G | -     | S | I | -     | S | S | -     | S | S |

(e)

**Figure A.7: Impact of stress on Shiga toxin-producing *Escherichia coli* (STEC) O111**

Growth (G), intermediate growth (I), or susceptibility (S) of STEC O111 by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 595 nm.

|         | 5 ppm |   |   | 10 ppm |   |   | 15 ppm |   |   | 17.5 ppm |   |   | 20 ppm |   |   | 22.5 ppm |   |   | 25 ppm |   |   | 27.5 ppm |   |   | 30 ppm |   |   |
|---------|-------|---|---|--------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|----------|---|---|--------|---|---|
| REP →   | 1     | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 | 1        | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G     | - | - | G      | - | - | G      | - | - | -        | G | I | G      | G | G | -        | S | I | S      | S | S | -        | S | S | S      | S | S |
| ACID    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | I | G | S      | S | S | -        | S | S | S      | S | S |
| ALK     | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | I | G | -        | S | G | S      | S | S | -        | S | S | S      | S | S |
| FT      | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | S | I | S      | S | S | -        | S | S | S      | S | S |
| HEAT    | G     | - | - | G      | - | - | G      | - | - | -        | G | G | G      | G | G | -        | I | I | S      | S | S | -        | S | S | S      | S | S |
| SALT    | G     | - | - | G      | - | - | I      | - | - | -        | G | I | G      | G | G | -        | S | I | S      | S | S | -        | S | S | S      | S | S |

(a)

|         | 0.50% |   |   | 0.75% |   |   | 1.00% |   |   | 1.25% |   |   | 1.50% |   |   | 1.75% |   |   | 2.00% |   |   | 2.25% |   |   | 2.50% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | I | G | I     | S | G | -     | S | G | -     | S | G | -     | S | G |
| ACID    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | S     | S | G | -     | S | G | -     | S | G | -     | S | G |
| ALK     | G     | - | - | G     | - | - | G     | - | - | G     | I | G | G     | I | G | S     | S | G | -     | S | G | -     | S | G | -     | S | G |
| FT      | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | I | G | S     | S | G | -     | S | G | -     | S | G | -     | S | G |
| HEAT    | G     | - | - | G     | - | - | G     | - | - | G     | G | S | G     | I | G | I     | I | S | -     | S | S | -     | S | S | -     | S | S |
| SALT    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | I | G | S     | S | G | -     | S | G | -     | S | G | -     | S | S |

(b)

|         | 90 ppm |   |   | 100 ppm |   |   | 110 ppm |   |   | 120 ppm |   |   | 130 ppm |   |   | 140 ppm |   |   |
|---------|--------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|---------|---|---|
| REP →   | 1      | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 | 1       | 2 | 3 |
| CONTROL | G      | G | G | G       | G | G | G       | G | I | G       | I | I | G       | S | S | S       | S | S |
| ACID    | G      | G | G | G       | G | G | G       | G | G | S       | I | I | S       | S | S | S       | S | S |
| ALK     | G      | I | G | G       | G | G | G       | I | G | S       | I | I | G       | S | S | I       | S | S |
| FT      | G      | G | G | G       | I | G | G       | I | G | S       | I | I | G       | S | S | S       | S | S |
| HEAT    | G      | G | G | S       | G | G | S       | S | I | S       | S | S | S       | S | S | S       | S | S |
| SALT    | G      | I | I | G       | I | G | G       | I | G | I       | S | S | I       | S | S | S       | S | S |

(c)

|         | 0.125% |   |   | 0.250% |   |   | 0.500% |   |   | 0.750% |   |   | 1.000% |   |   | 1.250% |   |   |
|---------|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|--------|---|---|
| REP →   | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 | 1      | 2 | 3 |
| CONTROL | G      | - | - | G      | G | G | G      | G | G | S      | S | I | S      | S | S | S      | S | S |
| ACID    | G      | - | - | G      | G | G | G      | G | G | S      | S | G | S      | S | S | S      | S | S |
| ALK     | G      | - | - | G      | G | G | G      | G | G | S      | S | I | S      | S | S | S      | S | S |
| FT      | G      | - | - | G      | G | G | G      | I | G | S      | S | I | S      | S | S | S      | S | S |
| HEAT    | G      | - | - | G      | G | G | G      | G | G | S      | S | I | S      | S | S | S      | S | S |
| SALT    | G      | - | - | G      | G | G | G      | I | G | S      | S | G | S      | S | S | S      | S | S |

(d)

|         | 0.05% |   |   | 0.10% |   |   | 0.20% |   |   | 0.30% |   |   | 0.40% |   |   | 0.50% |   |   | 0.60% |   |   | 0.70% |   |   | 0.80% |   |   |
|---------|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|-------|---|---|
| REP →   | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 | 1     | 2 | 3 |
| CONTROL | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | G     | I | G | -     | S | G | -     | S | I | -     | S | S |
| ACID    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | G     | I | G | -     | S | G | -     | S | S | -     | S | S |
| ALK     | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | G     | I | G | -     | S | G | -     | S | I | -     | S | I |
| FT      | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | G     | I | G | -     | S | G | -     | S | I | -     | S | S |
| HEAT    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | G     | I | G | -     | S | G | -     | S | G | -     | S | S |
| SALT    | G     | - | - | G     | - | - | G     | - | - | G     | G | G | G     | G | G | I     | I | G | -     | S | G | -     | S | S | -     | S | S |

(e)

**Figure A.8: Impact of stress on Shiga toxin-producing *Escherichia coli* (STEC) O145**

Growth (G), intermediate growth (I), or susceptibility (S) of STEC O145 by various antimicrobials. Antimicrobials are (a) LaurArg (lauric arginate), (b) CA+HCl (citric acid and hydrochloric acid), (c) PAA+AA (acetic acid, peroxyacetic acid, and hydrogen peroxide), (d) LA+CA (lactic acid and citric acid), and (e) LA (lactic acid). Alk=alkaline stress; FT=freeze-thaw stress; ppm=parts per million. Absorbance readings  $\geq 0.200$  were considered “growth”, readings  $< 0.200$  and  $> 0.100$  were considered “intermediate growth”, and readings  $\leq 0.100$  were considered “susceptible” all at 595 nm.

## Appendix B - Sample SAS Code for Analysis of MICs and Absorbance Values

```
data montalpha;
input Rep Conc $ Stress $ Abs;
datalines;
1      A      Acid 1.018
1      B      Acid 0.545
1      C      Acid 0.215
[data abbreviated]
3      D      Salt 0.077
3      E      Salt 0.076
3      F      Salt 0.03
;
run;

proc mixed data=montalpha;
class rep conc stress abs;
model abs=rep / ddfm=satterth;
TITLE 'rep variability';
run;

proc mixed data=montalpha;
class rep conc stress abs;
model abs=conc stress conc*stress / ddfm=satterth;
title 'Model w/out Rep as a random variable';
run;

proc mixed data=montalpha;
class rep conc stress abs;
model abs=conc stress conc*stress / ddfm=satterth;
lsmeans conc / pdiff cl;
title 'LSMEANS for Significant Variables';
run;

proc mixed data=montalpha;
class rep conc stress abs;
model abs=conc stress conc*stress / ddfm=satterth;
lsmeans conc*stress;
title 'LSMEANS Conc*Stress Abs Values';
run;
```

## Appendix C - SAS Code for STEC Attachment Study

```
proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE*MEAT
TIME*MEDIA*SAMPLE*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*MEAT*TEMP TIME*MEDIA*SAMPLE
TIME*MEDIA*MEAT TIME*MEDIA*TEMP
MEDIA*SAMPLE*MEAT MEDIA*SAMPLE*TEMP
MEDIA*MEAT*TEMP SAMPLE*MEAT*TEMP
TIME*MEDIA TIME*SAMPLE TIME*MEAT
TIME*TEMP MEDIA*SAMPLE MEDIA*MEAT
MEDIA*TEMP SAMPLE*MEAT SAMPLE*TEMP
MEAT*TEMP MEDIA SAMPLE MEAT TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Full Model';
run;
```

```
data TSB; set one;
if MEDIA='M' then delete;
run;
```

```
data M9; set one;
if MEDIA='T' then delete;
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
SAMPLE*MEAT*TEMP SAMPLE*MEAT*TIME
MEAT*TEMP*TIME TIME*SAMPLE TIME*MEAT
TIME*TEMP SAMPLE*MEAT SAMPLE*TEMP
MEAT*TEMP SAMPLE MEAT TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'TSB Full Model';
run;
```

```
proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
SAMPLE*MEAT*TEMP SAMPLE*MEAT*TIME
MEAT*TEMP*TIME TIME*SAMPLE TIME*MEAT
TIME*TEMP SAMPLE*MEAT SAMPLE*TEMP
MEAT*TEMP SAMPLE MEAT TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Full Model';
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
SAMPLE*MEAT*TEMP SAMPLE*MEAT*TIME
MEAT*TEMP*TIME TIME*SAMPLE TIME*MEAT
SAMPLE*MEAT SAMPLE TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'TSB Backwards Elimination Model 1';
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
```

```
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
TIME*SAMPLE TIME*MEAT SAMPLE*MEAT
SAMPLE TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'TSB Backwards Elimination Model 2';
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE TIME*MEAT
SAMPLE*MEAT SAMPLE TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'TSB Backwards Elimination Model 3';
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE TIME*MEAT
SAMPLE*MEAT TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'TSB FINAL MODEL';
run;
```

```
proc mixed data=TSB;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE TIME*MEAT
SAMPLE*MEAT TIME/DDFM=SATTERTH;
RANDOM REP;
LSMEANS TIME*SAMPLE TIME*MEAT
SAMPLE*MEAT TIME / pdiff cl;
Title 'TSB FINAL MODEL';
run;
```

```
proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
SAMPLE*MEAT*TEMP SAMPLE*MEAT*TIME
MEAT*TEMP*TIME TIME*TEMP*SAMPLE
TIME*SAMPLE TIME*MEAT TIME*TEMP
SAMPLE*MEAT SAMPLE*TEMP MEAT*TEMP
SAMPLE MEAT TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Complete Full Model';
run;
```

```
proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=SAMPLE*MEAT*TEMP*TIME
MEAT*TEMP*TIME TIME*SAMPLE SAMPLE*TEMP
MEAT*TEMP MEAT TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Backwards Elimination Model 1';
run;
```

```
proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE SAMPLE*TEMP
MEAT*TEMP MEAT TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Backwards Elimination Model 2';
run;
```

```

proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE MEAT*TEMP MEAT
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Backwards Elimination Model
3';
run;

proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE MEAT
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'M9 Backwards Elimination Model
4';
run;

proc mixed data=M9;
CLASS time sample meat temp rep;
MODEL LOG=TIME*SAMPLE MEAT*TEMP MEAT
TIME/DDFM=SATTERTH;
RANDOM REP;
LSMEANS time / pdiff cl;
Title 'M9 FINAL MODEL';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE*MEAT
TIME*MEDIA*SAMPLE*TEMP
TIME*MEDIA*MEAT*TEMP
TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA*MEAT
TIME*MEDIA*TEMP MEDIA*SAMPLE*MEAT
MEDIA*SAMPLE*TEMP SAMPLE*MEAT*TEMP
TIME*SAMPLE*TEMP TIME*MEAT*TEMP
MEDIA*MEAT*TEMP TIME*SAMPLE*MEAT
TIME*MEDIA TIME*SAMPLE TIME*MEAT
TIME*TEMP MEDIA*SAMPLE MEDIA*MEAT
MEDIA*TEMP SAMPLE*MEAT SAMPLE*TEMP
MEAT*TEMP MEDIA SAMPLE MEAT TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Full Model';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE*MEAT
TIME*MEDIA*SAMPLE*TEMP
TIME*MEDIA*MEAT*TEMP
TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA*MEAT
TIME*MEDIA*TEMP MEDIA*SAMPLE*MEAT
MEDIA*SAMPLE*TEMP SAMPLE*MEAT*TEMP
TIME*MEAT*TEMP MEDIA*MEAT*TEMP
TIME*MEDIA TIME*SAMPLE TIME*MEAT
MEDIA*SAMPLE MEDIA*MEAT MEDIA*TEMP
SAMPLE*MEAT SAMPLE*TEMP MEAT*TEMP MEDIA
SAMPLE MEAT TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.8';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;

```

```

MODEL LOG=TIME*MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*MEAT*TEMP
TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA*MEAT
MEDIA*SAMPLE*MEAT MEDIA*SAMPLE*TEMP
SAMPLE*MEAT*TEMP TIME*MEAT*TEMP
TIME*MEDIA TIME*SAMPLE TIME*MEAT
MEDIA*MEAT MEDIA*TEMP SAMPLE*MEAT
SAMPLE*TEMP MEAT*TEMP MEDIA SAMPLE MEAT
TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.6';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*MEAT*TEMP
TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA*MEAT
MEDIA*SAMPLE*MEAT MEDIA*SAMPLE*TEMP
SAMPLE*MEAT*TEMP TIME*MEAT*TEMP
TIME*MEDIA TIME*SAMPLE TIME*MEAT
MEDIA*MEAT MEDIA*TEMP SAMPLE*MEAT
SAMPLE*TEMP MEAT*TEMP MEDIA SAMPLE MEAT
TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.6 w/out
5 way interaction';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*MEAT*TEMP
TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA
TIME*SAMPLE TIME*MEAT MEDIA*TEMP
SAMPLE*MEAT MEAT*TEMP MEDIA SAMPLE MEAT
TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.5';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA
TIME*SAMPLE TIME*MEAT MEDIA*TEMP
SAMPLE*MEAT MEAT*TEMP MEDIA SAMPLE MEAT
TEMP TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.5
without 4 way interaction';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*SAMPLE*MEAT*TEMP
MEDIA*SAMPLE*MEAT*TEMP
TIME*MEDIA*SAMPLE TIME*MEDIA
TIME*SAMPLE MEDIA*TEMP SAMPLE*MEAT
MEAT*TEMP MEDIA SAMPLE TEMP
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.4';
run;

proc mixed data=one;
CLASS time media sample meat temp rep;

```

```

MODEL LOG=TIME*MEDIA*SAMPLE TIME*MEDIA
TIME*SAMPLE SAMPLE*MEAT MEAT*TEMP MEDIA
SAMPLE TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.3';
run;

```

```

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA*SAMPLE TIME*MEDIA
TIME*SAMPLE SAMPLE*MEAT MEDIA SAMPLE
TIME/DDFM=SATTERTH;

```

```

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA TIME*SAMPLE MEDIA
TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.1';
run;

```

```

proc mixed data=one;

```

```

RANDOM REP;
Title 'Model with P less than 0.3
without meat*temp';
run;

```

```

proc mixed data=one;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA TIME*SAMPLE
SAMPLE*MEAT MEDIA TIME/DDFM=SATTERTH;
RANDOM REP;
Title 'Model with P less than 0.2';
run;
CLASS time media sample meat temp rep;
MODEL LOG=TIME*MEDIA TIME*SAMPLE MEDIA
TIME/DDFM=SATTERTH;
RANDOM REP;
LSMEANS time*media time*sample media
time / pdiff cl;
Title 'FINAL MODEL w/LS MEANS';
run;

```

## Appendix D - SAS Code for SBS-PAA Spinach Experiment

```

data salmTSA;
input Rep Day Treat $ Log;
datalines;
1      0      Control      6.65
1      0      SBSPAA       5.01
1      0      Water 5.70
1      0      Chlorine      5.48
[data abbreviated]
3      10     Control      6.10
3      10     SBSPAA       4.25
3      10     Water 5.22
3      10     Chlorine      5.15
;
run;

proc mixed data=salmTSA;
class rep day treat log;
model log=rep / ddfm=satterth;
TITLE 'rep variability';
run;

proc mixed data=salmTSA;
class rep day treat log;
model log=day treat day*treat /
ddfm=satterth;
title 'Model w/out Rep as a random
variable';
run;

proc mixed data=salmTSA;
class rep day treat log;
model log=day treat /
ddfm=satterth;
title 'Model w/out Interaction';
run;

proc mixed data=salmTSA;
class rep day treat log;
model log= treat day /
ddfm=satterth;
lsmeans treat day / pdiff cl;
title 'Final Model w/LSMEANS';
run;

```

```

data salmxlt4;
input Rep Day Treat $ Log;
datalines;
1      0      Control      5.95
1      0      SBSPAA       4.96
1      0      Water 5.24
1      0      Chlorine      5.18
[data abbreviated]
3      10     Control      6.06
3      10     SBSPAA       4.25
3      10     Water 4.84
3      10     Chlorine      4.77
;
run;

proc mixed data=salmxlt4;
class rep day treat log;
model log=rep / ddfm=satterth;
TITLE 'rep variability';
run;

proc mixed data=salmxlt4;
class rep day treat log;
model log=day treat day*treat /
ddfm=satterth;
title 'Model w/out Rep as a random
variable';
run;

proc mixed data=salmxlt4;
class rep day treat log;
model log=day treat /
ddfm=satterth;
title 'Model w/out Interaction';
run;

proc mixed data=salmxlt4;
class rep day treat log;
model log= treat / ddfm=satterth;
lsmeans treat / pdiff cl;
title 'Final Model w/LSMEANS';
run;

```