ABSTRACT

Title of Document: ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF *ENTEROCOCCCUS* SPP. AND *SALMONELLA* SPP. FROM CONVENTIONAL POULTRY FARMS TRANSITIONING TO ORGANIC FARMING PRACTICES

Erinna Lea Kinney, Master's of Public Health, 2009

Directed By: Dr. Amy R. Sapkota, Assistant Professor
Maryland Institute of Applied Environmental Health

This baseline study evaluated prevalence and antibiotic resistance of food-borne bacteria as conventional poultry facilities transition to organic practices. Poultry litter, feed, soil, water samples and poultry questionnaire responses were collected from 10 conventional and 10 organic-transitioning poultry houses from March to June 2008. *Enterococcus* spp. (n=260) and *Salmonella* spp. (n=100) isolates were identified to species level and antimicrobial susceptibility testing was performed using the Sensititre® system. Statistical analyses were performed using STATA 10. Prevalence of *Enterococcus* spp. on organic-transitioning and conventional poultry farms was 100%; and prevalence of *Salmonella* spp. was 100% and 40%, respectively. *Enterococcus* isolates from conventional poultry houses displayed significantly higher percentages of resistance for 9 antibiotic agents compared to organic-transitioning isolates. Conversely, *Salmonella*

spp. isolated from both conventional and organic-transitioning poultry houses exhibited similar antibiotic resistance patterns. Baseline findings suggest importance of poultry production practice in prevalence and antibiotic resistance patterns of food-borne bacteria.

ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF *ENTEROCOCCCUS* SPP. AND *SALMONELLA* SPP. FROM CONVENTIONAL POULTRY FARMS TRANSITIONING TO ORGANIC FARMING PRACTICES

By

Erinna Lea Kinney

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master's of Public Health
2009

Advisory Committee:
Dr. Amy R. Sapkota, Chair
Dr. Betty Dabney
Dr. Sam W. Joseph
Dr. Amir Sapkota

© Copyright by

Erinna Lea Kinney

2009

Dedication

I dedicate my graduate experience to the great people in my life who have supported my educational pursuits with continued guidance, faith, and encouragement.

Unequivocally

Acknowledgements

First and foremost I am thankful for my journey and the sacrifices of those before me both known and unknown. I give thanks to God for the peace, blessings and joy; As well as, the guidance and directional influences of the Creator in my life

I would like to say a special thank you to:

My advisor, Dr. Amy R. Sapkota, for her belief in my abilities, guidance, and support. To her, I am very grateful for every continuing support, patience and understanding in my development as a graduate student. I have been inspired by her intellect, confidence and fervor for environmental health. To express my appreciation for her as my advisor, mentor, and teacher is without saying and I am completely indebted to her for my extraordinary experience within MIAEH. I am sincerely blessed that our paths cross in that her example becomes my aspiration for my future endeavors.

Dr. Sam Joseph, Dr. Betty Dabney, Dr. Amir Sapkota, and Dr. Guangyu Zhang for their patience and willingness to serve on my advisory committee. I would also acknowledge the faculty and staff in the University of Maryland School of Public Health, for their instruction, thoughtfulness and acts of kindness. I would also like to thank the people who assisted the project: Our collaborators at Penn State: Dr. Mike Hulet, Terry Cravener and Denny Burns for their assistance in the collection of on-site samples; Andrew Kim, Zenas Chang, Norman Wang in processing; Dr. McDermott, Linda English, Peggy Carter, and Althea Glenn for their training, laboratory support, and encouragement at FDA/CVM. As a critical component in my research study, I would like to give appreciation to my funding source for the execution of this projection: Johns Hopkins Center for a Livable Future.

Lastly, I would like to thank my parents, Mr. Wesley and Sheila Kinney for their support both lovingly and spiritually. My twin sister, Erica, who's mirrored- a dual experience as a graduate student, has taught me invaluable lessons. My brother, Damon, whose straight-talk and no-nonsense approach to life, has taught me to be self-reliant. My fellow friends in life whose support was always appreciated and needed; Jeanette Stewart, Kismet Little Ethell Vereen, and Tiffany Onifade. A special thank you to my classmates and lab mates in Environmental Health, specifically, and at the UMD School of Public Health, in general, who has helped me along the way. Finally, I want to acknowledge my favorite affirmation which was a constant source of perspective and strength:

> God is not in you in the same sense that a raisin is in a bun. That is not unity. God is in you as the ocean is in a wave. The wave is nothing more nor less than the ocean expressing as a wave...God is an allness in which you exist as an eachness.
>
> *Eric Butterworth*

Table of Contents

Dedication ii
Acknowledgements iii
Table of Contents v
List of Tables viii
List of Figures x

Chapter 1: Introduction

I. Overview
A. Antimicrobials and Antimicrobial Resistance 2
B. Agricultural Systems and Antibiotic Resistance 3
C. Poultry Production and Antibiotic Resistance 5
D. Research Rationale 7

Chapter 2: Background

I. *Enterococcus*
A. Historical Perspective 10
B. Genus Description 11
C. Ecological Habitat and Distribution 12
D. Epidemiology and Pathogenicity 13
E. Antibiotic Resistance 14

II. *Salmonella*
A. Historical Perspective 16
B. Genus Description 16
C. Ecological Habitat and Distribution 17
D. Epidemiology and Pathogenicity 19
E. Antibiotic Resistance 21

III. Poultry Production in the United States 22

IV. Organic Farming and Poultry 25

V. Antibiotic Usage and Antibiotic Resistance in Agriculture 28

Chapter 3: Methodology

I. Site Description 32

II. Sample Collection 32

III. Poultry Farm Questionnaire: Environmental Indices 33

IV. Isolation
A. Isolation and Enumeration of *Enterococcus* spp. and Salmonella spp. from Water....34
B. Isolation *Enterococcus* spp. from Poultry Litter and Poultry Feed Samples....35
C. Isolation *Salmonella* spp. from Poultry Litter and Poultry Feed Samples....35

IV. Identification
A. Identification of *Enterococcus* spp. Recovered from Water, Poultry Litter and Poultry Feed Samples....36
B. Identification of *Salmonella* spp. Recovered from Water, Poultry Litter and Poultry Feed Samples....37

VI. Antimicrobial Susceptibility Testing....38

VII. Statistical Analysis....39

Chapter 4: Results

I. Poultry House Characteristics....42

II. Enterococcus
A. Prevalence of *Enterococcus* spp. 43
B. Antibiotic Resistance of *Enterococcus* spp.: Minimal Inhibitory Concentration....44
C. Antibiotic Resistance of *Enterococcus* spp.: Resistance Patterns....45

III. Salmonella
A. Prevalence of *Salmonella* spp. 46
B. Antibiotic Resistance of *Salmonella* spp.: Minimal Inhibitory Concentration....47
C. Antibiotic Resistance of *Salmonella* spp.: Resistance Patterns....47

IV. Environmental Factors....48
A. *Enterococcus*....49
B. *Salmonella*....50

Chapter 5: Discussion and Conclusions

I. Discussion....52
A. *Enterococcus*....54
B. *Salmonella* 55
C. Environmental Factors on Antibiotic Resistance in Poultry Houses 57

II. Limitations 58
III. Public Health Implications.....59
IV. Conclusions.....62

Glossary 102

Appendices

A. Poultry Farm Sampling Protocol.....103
B. Poultry Farm Questionnaire 110
C. Poultry Farm Protocol for Isolation of *Enterococcus* spp. from Environmental Samples 118
D. Poultry Farm Protocol for Isolation of *Salmonella* spp. from Environmental Samples 122

References.....125

Curriculum Vitae 151

List of Tables

Table 1: Clinical and Laboratory Standards Institute Interpretative Criteria for MIC determinations and breakpoints 63

Table 2: Most common *Salmonella* spp. serovars isolated from Humans 66

Table 3: Organic Poultry Production in top four states from 2000-2005 67

Table 4: Selected antimicrobials approved for us in broiler production 68

Table 5: Poultry House Demographics for study Conventional and Organic-transitioning poultry houses 69

Table 6: *Enterococcus* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms 70

Table 7: Species-specific characterization of *Enterococcus* spp. collected from conventional and organic-transitioning poultry farms 71

Table 8: Species-specific characterization of *Enterococcus* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms 72

Table 9: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus* spp. (n=260) collected from conventional and organic-transitioning poultry farms 73

Table 10: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus faecalis (*n=180) collected from conventional and organic-transitioning poultry farms 75

Table 11: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus faecium (*n=113) collected from conventional and organic-transitioning poultry farms 77

Table 12: *Salmonella* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms 79

Table 13: Species-specific characterization of *Salmonella* spp. collected from conventional and organic-transitioning poultry farms 80

Table 14: Species-specific characterization of *Salmonella* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms 81

Table 15: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Salmonella* spp. (n=120) collected from conventional and organic-transitioning poultry farms 82

Table 16: Multi-drug antibiotic resistance profiles of *Salmonella* spp. isolated from conventional and organic-transitioning poultry farm samples 84

Table 17: Correlation Table of Environmental Variables associated with Conventional and Organic-Transitioning Poultry Houses adjusted by Intra-Poultry House and Intra-Farm Variation 85

List of Figures

Figure 1: Photograph of Sensititre™ antimicrobial susceptibility testing system (Trek Diagnostic Systems, Westlake, Ohio) 86

Figure 2: U.S. boiler production from 1967-2007 87

Figure 3: U.S. Broiler Production by State in 2007, (number produced thousand) 88

Figure 4: Photographic depiction of typical conventional and organic poultry houses. 89

Figure 5: Organic food share market in 2005 90

Figure 6: Schematic of Vertical Integration within the Broiler Production Industry 91

Figure 7: Number of U.S. certified organic poultry animals, 1997-2005 92

Figure 8: Percentage of Expressed Susceptible, Intermediate, and Resistant *Enterococcus* spp. Isolates from Conventional Poultry Houses to a particular antibiotic 93

Figure 9: Percentage of Expressed Susceptible, Intermediate, and Resistant *Enterococcus* spp. Isolates from Organic-Transitioning Poultry Houses to a particular antibiotic 94

Figure 10: Percentage of Total *Enterococcus* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=291) 95

Figure 11: Percentage of *Enterococcus faecalis* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=118) 96

Figure 12: Percentage of *Enterococcus faecium* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=112) 97

Figure 13: Percentage of Expressed Susceptible, Intermediate, and Resistant *Salmonella* spp. Isolates from Conventional Poultry Houses to a particular antibiotic 98

Figure 14: Percentage of Expressed Susceptible, Intermediate, and Resistant *Salmonella* spp. Isolates from Organic-Transitioning Poultry Houses to a particular antibiotic 99

Figure 15: Percentage of Total *Salmonella* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=121) 100

Figure 16: Percentage of *Salmonella kentucky* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=61) 101

Chapter 1

Introduction

Chapter 1: Introduction

I. OVERVIEW

A. Antimicrobials and Antimicrobial Resistance

Since the mid-20th century, antimicrobials have been utilized in the protection of human and veterinary health worldwide. According to the Centers for Disease Control and Prevention (CDC), antimicrobial agents are defined as "drugs, chemicals, or other substances that either kill or slow the growth of microbes" (CDC, 2008a). The specificity of antimicrobials is characterized by target entities or organism (i.e. antibacterial drugs-bacteria; antiviral agents-viruses; antifungal agents-fungi; and anti-parasitic drugs-parasites) (CDC, 2008a). In tandem, the use of antimicrobials for the treatment of human and animal illnesses has revolutionized and eroded many advances of modern clinical and veterinary medicine. Antimicrobials have significantly contributed to the prevention (Nadelman et al., 2001) and treatment of infectious diseases in humans, as well as myriad animal species. However, the excess or overuse of antibiotics can generate genomic selective pressures to enable microbes to adapt and acquire resistance (Witte, 2000).

Antibiotic resistance is an evolutionary artifact of microbes adapting to environmental changes associated with both natural and anthropogenic stressors (Banquero, Negri, Morosini, & Blazquez, 1998). The use of antimicrobials selects for resistance genes in both pathogenic and non-pathogenic bacteria (Aarestrup, 1999). Due to the rapid reproduction rates of bacteria, resistance can emerge in occurrence with antimicrobial agents. Resistance genes can surface in the bacterial gene pool. Consequently, the elevated exposure to antimicrobials, especially at

chronic low levels, amplifies the pool of resistant bacteria; and increases potential risk of clinical infection exhibiting antimicrobial-resistance (Levy, 1998; van den Bogaard & Stobberingh, 1999).

Nodes of antibiotic resistance can emerge in a variety of settings, including hospitals (McGowan Jr., 1983), long-term facility (Strausbaugh, Crossley, Nurse, & Thrupp, 1996), community-acquired (S. Madhi, Peterson, A. Madhi, Khoosal, & Klugman, 2000) and within the environment (Kummerer, 2004). The emergence of antibiotic resistance subsequently compromises the efficacy of drugs, chemicals, or other agents in disease treatment and prevention that could herald the onset into a "Post Antibiotic Era", where the availability of effective antibiotics no longer exists. Ultimately, increases in bacterial antibiotic resistance pose a considerable threat to public health, especially for vulnerable populations including young children (Shea, 2003), the elderly (Nicolle, Strausbaugh & Garibaldi, 1996), and immunocompromised individuals (Hitti & Wolff, 2005).

B. Agricultural Systems and Antibiotic Resistance

A major practice that leads to the development of bacterial antimicrobial resistance is the use and misuse of antibiotics in agriculture systems worldwide (Levy, 2004; Smith, Harris, Johnson, Silbergeld, & Morris, 2002). Two diametric agricultural methods surround the issue of antibiotic usage in agricultural settings: 1) conventional farming methods that employ standard agricultural industry practices that involve the usage of pesticides, synthetic fertilizers, antibiotics and other agribusiness approaches; and 2) organic farming practices that involve the production of foodstuff grown or raised without synthetic fertilizers or pesticides, antibiotics,

chemicals or hormones. With the transition from small-scale traditional farming to large-scale, concentrated animal feeding operations (CAFOs) that has occurred in the past century, agricultural practices have evolved to accommodate food consumption rates with increased agricultural output at the risk of introducing antibiotic-resistant pathogens into the environment (Silbergeld, Graham, & Price, 2008).

In the U.S., annual estimates state that between 9 and 13 million kg of antibiotics are used for agricultural purposes (Shea, 2003). Four key functions of antibiotics in agriculture involve: 1) therapeutic--treatment of sick animals; 2) metaphylaxis--short-term treatment for diseased animals and prevention of spread of disease; 3) prophylactic--prevention of infection or development of disease; and 4) growth promotion-- enhancement of weight gain and feed efficiency conversion—the amount of food converted to animal protein rather than manure (Mathew, Cissel, & Liamthong, 2007; Pew Commission, 2008). Upwards of 70% of antibiotics commercially produced in the United States are employed non-therapeutically in animal agriculture to promote farm animal growth (Mellon, C. Benbrook, & K. Benbrook, 2001). Many of the antibiotics used in food animal operations are human therapeutic equivalents or analogs in clinical medicine where some antibiotic usage is the only form of treatment. For example, Synercid, a combination of quinupristin and dalfopristin, is a third- generation antibiotic of the streptogramin class approved by FDA for human use in the U.S. (FDA, 1999). Synercid is the first antibiotic approved for the treatment of vancomycin-resistant *Enterococcus faecium* (VREF) infections in the presence of no alternative treatment in the U.S. (FDA, 1999). In addition, approval had been issued for use in the treatment of complicated skin and skin structure infections (FDA, 1999). Economic additional cost estimates to the U.S. healthcare system due to

antibiotic-resistant bacteria range from 4-5 billion dollars per year (Harrison, 1998). In addition, several studies have suggested that characteristics of agricultural environmental settings, including animal crowding, CAFO hygiene, temperature, ventilation control, and stress, can influence antibiotic resistance and pathogen risk (Gilchrist et al., 2007).

C. Poultry Production and Antibiotic Resistance

Intensive animal feeding production of poultry can potentially provide a suitable environment for the proliferation of antibiotic resistant bacteria (Hayes et al., 2001 Hayes et al., 2003; Price, Lackey, Vailes, & Silbergeld, 2007). Between 1945-1999, the poultry production industry experienced an 88 % increase in the production of broiler chickens in billion pounds per year (USDA, 2006). An estimated 10.3 million pounds of antibiotics are used annually in U.S. poultry production for non-therapeutic purposes such as promoting growth and improving feed efficiency with a 307% per bird antimicrobial use increase since the 1980s (Mellon et al., 2001). A host of antibiotics are approved by the FDA for growth promotion in poultry production Table 1.

Predominantly outside of the U.S., numerous studies have reported the use of antibiotics in poultry production as a causative agent in the establishment of antibiotic-resistance reservoirs within poultry flocks (Bager, Madsen, Christensen, & Aarestrup, 1997; Hayes et al., 2001 Hayes et al., 2004; Singer & Hofacre, 2006; Price et al., 2007). Significant associations between the use of non-therapeutic antimicrobials and antibiotic resistance among pathogenic and non-pathogenic bacteria have been documented in several studies investigating *Campylobacter* spp., *Salmonella* spp. and *Enterococcus* spp. recovered from conventional and organic farming

operations (Langtongkum et al., 2005; Gupta et al., 2004; Garcia-Migura, Pleydell, Barnes, Davies, & Liebana, 2005). In recent work, research has exhibited a myriad of resistance patterns ranging between an elevation (Angkititrakul, Chomvarin, Chaita, Kanistanon, & Waethewutajarn, 2005; Louge, Sherwood, Olah, Elijah, & Dockter, 2003), a reduction (Cui, Ge, Zheng, & Meng, 2005; Price, Johnson, Vailes, & Silbergerd, 2005), and no effect (Joseph, in press) in percent of antibiotic-resistant bacteria within organic and conventional farming operations, comparatively.

With the meteoric rise of the organic market (i.e. organic meat is the fastest growing sector of the organic market share (Dimitri & Greene, 2002)), new interest is arising with regard to conventional farms transitioning to organic practices to capitalize commercially from the organic niche (Olberholtzer, Dimitri, & Greene, 2006). However, as these transitions to organic practices occur on poultry farms, there is a paucity of knowledge concerning on-farm temporal changes associated with antibiotic resistance and food borne bacteria. Limited knowledge also exists in elucidating the role of environmental factors on long-term antibiotic-resistant patterns and prevalence of food-borne bacteria on poultry farms. No previous or comparable studies have been fully assessed in the United States. Thus, the primary objective of this study was to conduct a prospective, longitudinal, on-farm investigation in the United States to evaluate temporal changes in antibiotic resistance and loads of *Salmonella* spp. and *Enterococcus* spp. in association with the implementation of organic poultry production practices, and to further evaluate how other environmental factors may modify this association.

D. Research Rationale

In the present study, we built upon the work of previous cross-sectional studies to examine the prevalence of pathogenic/non-pathogenic bacteria and the patterns of antibiotic-resistant bacteria on conventional poultry farms and farms transitioning to organic practices. We also sought to evaluate the influence of environmental factors on pathogen prevalence and antibiotic resistance. *We hypothesized that gradual changes in microbial loads and phenotypic antibiotic resistance of Salmonella spp. and Enterococcus spp. would occur on organic-transitioning poultry farms over time. In addition, we hypothesized that associations would exist between environmental covariates and on-farm prevalence and phenotypic resistance in food-borne bacteria in poultry production environments.* The specific aims of the study include the following:

1. To characterize prevalence and antibiotic resistance of *Salmonella* spp. and *Enterococcus* spp. recovered from the same poultry farms over time as the farms convert from conventional to organic practices and discontinue the use of antibiotics

2. To quantify temporal changes in on-farm antibiotic resistance and carriage of antibiotic resistance genes in *Salmonella* spp. and *Enterococcus* spp. during conversion process

3. To evaluate associations over time between on-farm levels and genotypic antibiotic resistance of *Salmonella* spp., and *Enterococcus* spp. and an array of environmental variables.

The work completed for this master's thesis serves as the baseline data for the long-term prospective, on-farm study of microbial pathogen load and antimicrobial susceptibility patterns of commensal (*Enterococcus* spp.) and pathogenic (*Salmonella* spp.) microorganisms associated

with the transitioning of large-scale conventional poultry farms to organic agricultural poultry production practices.

Chapter 2

Background

Chapter 2: Background

I. ENTEROCOCCUS

A. Historical Perspective

The genus of *Enterococcus* was first documented in 1899 by Thiercelin (Thiercelin, 1899) as *entérocoque* – a reference to its intestinal source and appearance as pairs or short chains in human feces. Later described in clinical cases of endocarditis (MacCallum & Hastings, 1899) and via environmental isolation from sewage (Kühn et al., 2000), *Enterococcus* was broadly described as "streptococcus of fecal origin". Subsequent organisms of the streptococcal genus were identified based on fermentation activity: *Streptococcus faecalis* (Andrews & Horder, 1906); *Streptococcus faecium* (Orla-Jensen,1919); and *Streptococcus durans* (Sherman, 1937). In 1937, a streptococci taxonomical system was developed to represent the following categories: pyogenic, lactic, viridians, and enterococcus. The "*enterococcus*" group corresponded with streptococci that grew 1) at temperatures ranging from 10°C to 45°C, 2) at an adjusted pH of 9.6 and 3) at 6.5% NaCl. These organisms also could survive temperatures upwards of 60°C for 30 min and had the ability to split esculin (Sherman, 1937). Within the *Enterococcus* group, members correlated with the Lancefield serological scheme that reacted with group D antisera were commonly referenced as Group D Streptococcus (Murray, 1990).

Moreover, lesser known species of the *Enterococcus* group have been isolated from human, animal, plant and food origins. Motile enterococci have been acknowledged since the early 1930's (Motarjemi & Adams, 2006). A Gouda cheese-derived *enterococcus* was described as "malodoratus" due to its pungent smell in 1955 and later termed *S. faecalis* var.

malodroatus (Murray, 1990). Another notable addition, pigmented enterococci, were identified in the 1950s and a designation of *S. faecium* var. *casselifavus* (for yellow color) was later suggested in 1968 (Murray, 1990). Nowlan and Deibel identified *Streptococcus avium* from poultry in 1967 (Murray, 1990). Two watershed events ushered a taxonomical challenge for *Enterococcus*. In 1970, Kalina recommended a separate genus for the enterococcal streptococci and the reassignment of *S. faecalis, S. faecium,* and subspecies of *Enterococcus* based on cellular arrangement and phenotypic associations; however, the proposal was largely disregarded and the classification as Group D Streptococcus persisted until 1984. With the advent of DNA hybridization and phylogenetic analysis, a separate genus classification of *Enterococcus* was warranted (Schleifer & Kilpper-Balz.1984) due to significant genetic distances of *S. faecalis* and *S. faecium* to other streptococci. To date, the current genus classification of *Enterococcus* is valid and generally accepted within the microbiological community.

B. Genus Description

Members of the genus *Enterococcus* include gram positive, facultatively anaerobic cocci that are ovoid in form and can occur in singlet, pairs or short chains (Facklam, 2002). *Enterococcus* spp. are homofermentative lactic acid bacteria that lack cytochrome enzymes (Murray, 1990). In biochemical screens, *Enterococcus* spp. normally exhibit catalase-negative properties; yet some strains produce pseudocatalase and can appear to be weakly catalase-positive (Murray, 1990). The characteristic attributes of *Enterococcus* spp. include growth at 1) temperatures ranging from 10°C to 45°C, 2) an adjusted pH of 9.6 and 3) 6.5% NaCl, and survival in temperatures upwards of 60°C for 30 min (Murray, 2008). *Enterococcus* also has the

ability to hydrolyze esculin in the presence of 40% bile salts (Sherman, 1937). In addition, serological determinations involve reaction with group D antisera and limited reaction with group Q antisera (Murray, 1990). Hydrolysis of pyrrolidonlyl-β-naphthylamide (PYR) is proficient in most representative species of enterococci. Only two species, *Enterococcus. casseliflavus* and *Enterococcus gallinarium*, demonstrate motility capabilities (Facklam, 2002). *Enterococcus* spp. can express alpha, gamma, or beta hemolysis on blood agar (Levinson, 2006). Since the transfer of *S .faecalis* and *S. faecium* from the genus Streptococcus to create the genus *Enterococcus*, the present number of total enterococci species is 26 based on chemotaxonomic and phylogenetic analysis (Schleifer, 1984).

C. Ecological Habitat and Distribution

Enterococci reside in the microbial environment of the intestines and various species can be isolated from nearly all mammals, in particular humans (Murray, 1990; Facklam, 2002). To a lesser degree, enterococci exist in non-mammal reservoirs such as reptiles, birds, fish, insects and even plant communities (Aaerstrup, 1999). As a result, *Enterococcus* spp. are ubiquitous in the natural environment and can be recovered from various environmental media: soil (Mundt, 1961), air (Chapin, Rule, Gibson, Buckley, & Schwab, 2005), water (Rice, Messer, Johnson, & Reasoner, 1995), and food (Giraffa, 2002). Remarkably robust and resilient, *Enterococcus* spp. can tolerate a wide array of environmental conditions such as high temperatures and high pHs that would normally inhibit or kill most microorganisms (Hardie, 1986). In humans, enterococci comprise only 1% of the enteric microflora, but are characterized by an unexpected spectrum of species diversity (Tannock & Cook, 2002). Species composition and dominance vary within the

intestines and across geographical landscapes (Blanch et al., 2003). In some parts of the world, *E. faecalis* exerts antibiotic resistance prominence. For example, Manson, Keis, Smith, & Cook (2003) attributed the presence of a clonal lineage of VanA-type *E. faecalis* which dominates in vancomycin-resistant *Enterococcus* (VRE) isolated from poultry and humans in New Zealand. The specific ecological and/or microbiological mechanisms promoting such selection of intestinal colonization are largely unknown (Murray, 1990).

D. Epidemiology and Pathogenicity

Enterococci are commensal bacteria with notable recognition as opportunistic pathogens of increasing public health importance (Huycke, Sahm, & Gilmore, 1998). Regarded as a minimal-grade pathogen, *Enterococcus* spp. were historically considered of nominal clinical impact, primarily affecting immunocompromised and sensitive individuals (Murray, 1990). Since the late 1990's, *Enterococcus* spp., however, have emerged in clinical significance as a leading cause of nosocomial or hospital-acquired secondary infections (Weinstein, 1998). *E. faecalis* and *E. faecium* are the most prevalent enterococci isolated from clinical human *Enterococcus* infections, accounting for 80-90% and 15-20% of infections, respectively. Enterococci-mediated nosocomial infections is the third most common cause of nosocomial infections in the United States (Schaberg, Culver, & Gaynes, 1991) resulting in approximately 1 out of every 8 hospital-acquired infections each year (CDC, 2008b). In addition, enterococci are the leading cause of surgical-site infections and the third leading cause of bloodstream sepsis infections (Richards, Edwards, Culver, & Gaynes, 2000) and are implicated in bacterial endocarditis, intraabdominal infections, bacteremia, and meningitis (Huycke et al., 1998).

Specifically, *Enterococcus* is also directly associated with approximately 110,000 urinary tract infections, 25,000 bacteremias, 40,000 wound infections, and 1,100 cases of endocarditis yearly in the United States (Huckye, 1998). Select risk factors associated with the acquisition of nosocomial enteroccocal infections include: gastrointestinal colonization, prior underlying conditions, prolonged hospital stays, transplantation, and prior antibiotic treatment (ODH-IDCM, 2007).

The pathogenicity of *Enterococcus* spp. entails the general colonization of mucosal surfaces with these organisms (Johnson, 1994). Presently, *Enterococcus* employs a variety of the microbial mechanisms for the colonization and factors that influence colonization of one species over another in the intestinal tract are also not well understood (Jett, Huycke, & Gilmore, 1994). However, it is known that pathogenicity of the bacterium involves a number of steps: a. secretion of cytolysins and other toxins that breach cellular membranes, b. evasion of host immune system defenses, and c. adherence factors (Murray, 1990).

E. Antibiotic Resistance

Virulence of *Enterococcus* is heavily coupled with their inherent or acquired resistance to antibiotics (Mundy, Sahm, & Gilmore, 2001). As defined by the World Health Organization (WHO), antibiotic resistance is "a natural biological phenomenon which can be amplified or accelerated by a variety of factors; where microbes over time acquire the ability to adapt to concentrations of antimicrobial agents" (WHO, 2002). Inherent resistance is a genetically mediated resistance to antibiotics which is non-transferable (Murray, 1998). Most *Enterococcus* spp. are innately resistant to cephalosporins and the semi-synthetic penicillinase-resistant

penicillins (e.g., oxacillin), many Beta-lactomases and low levels of aminoglycosides (Murray, 1998). Acquired resistance in *Enterococcus* is a result of the mutation of existing DNA (i.e. mutations) or the conference of resistance via genetic elements such as transposons and or plasmids. *Enterococcus* spp. are genetically "promiscuous" and easily exchange resistance-encoding genes to other *Enterococcus* or potentially more virulent non-*Enterococcus* species, i.e. *Staphylococcus aureus* (Noble, Virani, & Cree, 1992). Studies have documented the transfer of antibiotic resistance genes across species' ranges (Kuhn et al., 2000). As a result, multi-drug resistant (MDR) *Enterococcus* has steadily increased in the last two decades (Huycke et al., 1998). Enterococci acquired gentamicin resistance in 1979 (Eliopoulos, 1988) followed, in tandem, by penicillin resistance (Murray, 1990) and β lactamase resistance (Murray, 1990) during the early 1980's. Vancomycin represents one of the last antimicrobial strongholds against enteroccocal infections (Morris et al., 1995). However, vancomycin resistant *Enterococcus* or VRE has become a significant public health concern (CDC, 2008b). Increased incidence of VRE infections have ballooned twenty-fold from 1989-1993 in the US (MMWR, 1993). The incidence of hospitalizations with vancomycin-resistant *Enterococcus* increased from 4.60 to 9.48 hospitalizations per 100,000 people during 2003–2006 (Ramsey, 2009). The relative risk of death associated with antibiotic-resistant enterococci is several folds higher than that of susceptible enterococci (Edmund, Ober, Weinbaum, & Wenzel, 1996). Thus, the issue of antibiotic-resistant enterococci, specifically, VRE, has surfaced as an escalating public health hazard, in the United States and globally.

II.SALMONELLA

A. Historical Perspective

The discovery of *Salmonella* is well documented in recorded history. As early as the mid-1800s, scientific interest in *Salmonella* was initiated by the organism's potential etiological associations with typhoid fever (Cunha, 2004). *Salmonella* was first alluded to as a source of infection in typhoid fever patients following the confirmation of typhoid transmission via the fecal-oral route by William Budd in 1873 (Ellermeier & Slauch, 2006). Karl Eberth (1835-1926) noted rod-shaped organisms in the lymph nodes and spleens of typhoid patients in 1880 (Ellermeier & Slauch, 2006). The first successful cultivation of *Salmonella* serovar Typhi was performed by George Gaffky (1850-1918) from German patients in 1884 (Ellermeier & Slauch, 2006). Concurrently, a non-human Salmonella *spp., Salmonella choleraesius,* was isolated from a swine's intestine by Theobald Smith (1859-1934) under the direction of Daniel E. Salmon (1850-1914) in 1885 (Ellermeier & Slauch, 2006). In 1890, the term "*Salmonella*" became the established moniker for subsequent representative bacteria of the genera in tribute to the scientific contribution of Salmon (Ellermeier & Slauch, 2006).

B. Genus Description

Members of the genus *Salmonella* are Gram-negative, motile, facultatively anaerobic, bacilli belonging to the family *Enterobacteracae* (Ellermeier & Slauch, 2006). *Salmonella* are comprised of two central species, *Salmonella enterica* and *Salmonella bongori.* The nomenclature of *Salmonella* species has been marred by the traditional method of recognizing different serovars of *Salmonella* as distinct species (Brenner et al., 2000). Presently, six

subdivisions of *Salmonella enterica* subspecies I-VI exist with over 2500 serovars currently identified and several common serovars to human clinical infections (Table 2). Serovars are distinguished by their flagellar protein (H), lipopolysaccharide (O), carbohydrate composition and at times capsular (Vi) antigen (Coburn, 2007). Strains associated with subspecies I indicate sources of human and warm-blooded origin. Whereas, the remaining subdivisions, as well as *S. bongori,* usually originate from the environment and ectoderms. Typical cell morphology is rod shaped bacteria with cell size varying from 0.7-1.5 µm by 2-5 µm with growth conditions ranging from 7-48°C-- optimal growth for *Salmonella* spp. is at 37C (Ellermeier & Slauch, 2006). *Salmonella* spp. are neutrophiles with optimal growth at pH 6.5-7.5 (Ellermeier & Slauch, 2006). In addition, a water activity (Aw) level of 0.995 is ideal for maximum *Salmonella* growth (Cox, 1999). Prior research has determined that surface litter A_w levels greater than 0.90 appear to be predictive of *Salmonella* contamination in broiler and roaster houses (Carr et al., 1995). Characteristically, *Salmonella* spp. exhibits hydrolysis of 4-methylumbilliferyl caprylate (MUCAP), production of hydrogen sulfide, and the inability to metabolize lactose or sucrose (Ruiz, 1996). For the remainder of this document, *Salmonella* species will be referred to in their annotated form, i.e., *Salmonella ser. Typhimurium*→ *S. typhimurium.*

C. Ecological Habitat and Distribution

Salmonella spp. are ubiquitous in nature with a widespread geographical and epidemiological distribution (Porwollik, 2002). The internal habitat of *Salmonella* is mainly

resigned to the intestinal region of animals (Ellermeier & Slauch, 2006). *Salmonella* spp. can be isolated from nearly every environmental reservoir including soil (Baloda, Suraj, Christensen, & Trajcevska, 2001), vegetation (Beuchat, 2002), water (Yu & Bruno, 1996), animal (Jones, 1976) and food (White et al., 2001). The distribution of *Salmonella* can vary greatly depending on the serovar. Generalist species such as *Salmonella ser. Enteritidis* and *Salmonella ser. Typhimurium* have established global niches (Ellermeier & Slauch, 2006). However, many host-restrictive serovars are bound to specific geographical regions (Uzzau et al, 2000). Salmonella is found routinely where intensive agriculture practices are prevalent. From an agricultural perspective, *Salmonella* spp. are regularly isolated from infected food-producing animals, animal feeds, animal foodstuffs, especially of milk-, meat- or egg origin, and even within the farm environment as a reservoir. With optimal growth at 37°C, *Salmonella*, specifically, *S. enterica* is well suited for growth in warm-blooded animals. In contrast, all other S. enterica subspecies and *S. bongori* exhibit a commensal relationship with poikilotherms or cold-blood animals, i.e. reptiles, amphibians or fish (Ellermeier & Slauch, 2006). The serovar group *S. enterica* is further categorized by host range (Ellermeier & Slauch, 2006). The three main host range classifications include: a. host adapted: infection of one host but with the capacity to cause disease in others; b. host-restricted: infection of only a single host; and c. generalist: infection of a plethora of animals but disease manifestation varies in different hosts (Uzzau et al. 2000; Edwards, Olsen, & Maloy, 2002). For example, *S. typhi* is a host-restricted human pathogen (Ellermeier & Slauch, 2006). In many reptile and avian species, *Salmonella* infection is endemic but usually asymptomatic (Seepersadsingh & Adeisyun, 2003). Vehicles for continued persistence in the environment include a. fecal shedding of symptomatic and

symptomatic animals; b. contact with *Salmonella*-infected individuals, c. ingestion of contaminated food products (Winfield & Groisman, 2003). Studies have isolated the presence of *Salmonella typhimurium* and *Salmonella dublin* in the environment in excess of a year (Humphrey, 2001). The ecological resilience of Salmonella can be credited to their pathogenic diversity which promotes its evolution with their hosts and in the environment (Bäulmer, Tsolis, Ficht, & Adams, 1998).

D. Epidemiology and Pathogenicity

Salmonella is a leading cause of food-borne mortality and morbidity in the United States (MMWR, 2008). The clinical manifestation of *Salmonella* infection presents as Salmonellosis, an enteric condition which ranges in severity from self-limiting gastroenteritis to septicemia (Shere et al., 1998). The severity of disease depends heavily on host susceptibility and the virulence of the serovar. All serovars can produce all forms of salmonellosis; however, a given serovar is usually syndrome-specific (Ellermeier & Slauch, 2006). For example, *Salmonella choleraesuis* is normally associated with septicemia (Uzzau et al., 2001). *Salmonella* asymptomatic carriage is experienced in five percent of the human population (Perreten et al., 2005). With correspondence to disease outcome, strains of *Salmonella* are grouped as typhoidal and non-typhoidal organisms (Ellermeier & Slauch, 2006). An estimated 1.4 million cases per year of gastroenteritis and enteric fever in the United States can be attributed to non-typhi *Salmonella* (Mead et al., 1999). Accounting for 10% of food-borne illness in the U.S., the incidence of Salmonellosis occurs at a rate of 16.20 cases per 100,000 a year (MMWR, 2008). With respect to food-borne illnesses, Salmonellosis, contributes to 26% of the hospitalizations (

~15,000 /year) and 31 % of deaths (400-600 deaths/year) (Voetsche et al. 2004). Transmission of *Salmonella* spp. occurs via the fecal-oral route with fomites and insect vectors, i.e. flies, also playing major roles in the spread of *Salmonella* (Mian, 2002). Vertical transmission of *Salmonella* spp. has been documented in avian species via the infection of the vitelline membrane, albumen and potentially egg yolk (Berchieri Jr., 2001). In mammalian species, *in utero* transmission of *Salmonella* has been confirmed (Ault, Kennedy, Seoud, & Reiss,1993). *Salmonella* exhibits a seasonal pattern with the highest incidence of attributable food-borne illness occurring during the summer (D'Souza et al., 2004).

The majority of isolates that cause disease in humans and animals are classified under the *S. enterica* category (Brenner et. al., 2000). In 2007, the most common human-derived serovars were *S. enteritidis* and *S. typhimurium*, making up, in total, 36% of confirmed cases in the United States (MMWR, 2008). Other important serovars associated with human infections include *S. typhi*, *S. paratyphi* and *S. hirshfeldii* . Infections from specific human pathogens, serovars Typhi and Paratyphi, result from the ingestion of fecal-contaminated consumables, e.g. food or water and contact with current or chronic carriers of typhoid fever (Miller & Pegues, 2000). Due to elevated standards of water and waste sanitation, typhoid fever is relatively uncommon in the United States, approximately 400 cases per year (CDC, 2005). Over two-thirds of recognized cases in the United States result from international travel to endemic countries such as India or South Africa with incidence rates of 980 and 850 cases /100,000 per year, respectively (Bhan, Bahl, & Bhatnagar, 2005). Typhoid fever is a global health issue of eminent public health concern. Worldwide, typhoid affects over 21.6 million cases with a case-fatality rate ranging from 10-35% (Maskalyk, 2003; Crum, 2003).

The pathogenicity of *Salmonella* is a complex cascade of microbial and metabolic events. All salmonellae express virulence regardless of source and host status, e.g. carrier state (Ohl & Miller, 2001). Pathogenicity is mediated by certain factors such as strain virulence, infectious dose, route of infection, and host susceptibility (Groisman, Fields, & Heffron, 1990). M cells are the target cells of *Salmonella* pathogenicity (Ohl & Miller, 2001). If the proportion of vacant M cells to *Salmonella* spp. is high, manifestation of disease will occur. The status of the normal intestinal flora dictates target cell vacancy. Aberrations in the intestinal micro-environment, e.g. antimicrobial therapy, create suitable conditions for *Salmonella* to gain access to M cells (Ohl & Miller, 2001). Adhesion and assimilation into the M cell target are initial steps of *Salmonella* pathogenesis. After which, *Salmonella* are located in the submucsoal tissue and the lymph nodule. *Salmonella* excrete enterotoxins that create a toxin-induced response in the form of diarrhea and abdominal pain. Dissemination and reproduction of *Salmonella* spp. take place within phagocytic cells (Slauch et al., 1997); resulting in most gastroenteritis symptoms of *Salmonella* infection.

E. Antibiotic Resistance

Amongst *Salmonella* spp., antibiotic resistance is a well confirmed phenomenon. Animals are the primary source of zoonotic salmonella. Combined with intensive animal agricultural practices, including the therapeutic, prophylactic and non-therapeutic use of antibiotics, selection for antibiotic-resistant strains of *Salmonella* became inevitable. As early as the mid-1960s, outbreaks of resistant *Salmonella* spp. have been observed within animal

populations (Threlfall, 2002). *S Typhimurium* isolated from cattle has demonstrated resistance to ampicillin, chloramphenicol, kanamycin, neomycin, streptomycin, sulfonamides, tetracycline, and furazolidone (Threlfall, Ward, Frost, & Willshaw,2000). Multi-drug resistant *S. Typhimurium* DT104 consistently display antibiotic resistance patterns to five different antibiotics (Threlfall, 2000). Epidemic strains have exhibited resistance to approximately nine antibiotics (Baggesen, 2000). As reported by NARMS, 14.8% of non-Typhi *Salmonella* isolates were resistant to 2 or more Clinical and Laboratory Standards Institute (CLSI) subclasses of antibiotics and 7.6 % were resistant to 5 or more CLSI subclasses in 2005 (NARMS, 2005). With respect to *S. typhimurium*, 33.2% of isolates were resistant to 2 or more CLSI subclasses with 23% being resistant to 5 or more CLSI subclasses (NARMS, 2005). Recently, a multi-drug resistant strain of Salmonella enterica serovar Newport has become established in the U.S. and caused several outbreaks associated with retail meat and milk (Gupta et al., 2003). Genetic-relatedness of the *S. newport* U.S. strains has been detected in Japan giving credence to the global spread of multi-antibiotic resistance (Ishiguro, 2004).

III. POULTRY PRODUCTION IN THE UNITED STATES

The United States ranks as the world's leader in poultry production and second in egg production (USDA-ERS, 2009). The designation of "poultry" encompasses all domesticated fowl raised for the production of meat and eggs (USDA-ERS, 1999). The production of poultry includes the rearing of chickens, turkeys, ducks, geese; as well as more exotic species such as emus, ostrich and a variety of game birds. The geographic distribution of U.S. poultry

production is concentrated within four regions (Northeast, Appalachian, Delta, and Southeast) representing more than 70 percent of total U.S. poultry value (USDA-ERS, 1999). According to the Economic Research Service (ERS) (under the direction of the United States Department of Agriculture (USDA)), the on-farm value of U.S. poultry production totaled 31.9 billion in 2007 (USDA-ERS, 2007). Within the U.S. poultry industry, total production (farm value in dollars) was comprised of: broilers- $21.5 billion (67%); eggs -$6.68 billion (21%); turkey- $3.71 billion (12%); and others- $50.8 million (1%) in 2007 (National Ag Statistics Service (NASS), USDA, 2008). In particular, U.S. production of broilers, industry name for "young chickens raised exclusively for meat production", has steadily increased over the last two decades. Production of broilers exceeded over 8.05 billion pounds (Figure 2) in 2007, representing nearly 83% of the total birds produced that year. Top broiler producing states (in thousands of birds produced) include Georgia (1,398,800), Alabama (1,014,900) and Mississippi (824,000) (Figure 3) (USDA-NASS, 2006). Commercial demand has acted as a significant economic driver of U.S. poultry production with modifications of U.S. meat-consumption patterns beginning in the late 1960's (USDA-ERS, 1999). The consumption of poultry averaged 86 pounds per person in 2006, triple the 1960 consumption levels (USDA-ERS, 2008b). Dovetailing U.S. consumption patterns, demand for poultry worldwide has yielded a thriving avenue for exportation of U.S. poultry products (Windhorst, 2007). The United States dominates as the world's leader in exportation of poultry products with the European Union and the Russian Federation as major importers of U.S. poultry products in 2007 (AgMRC, 2009).

The enterprise of poultry production has evolved since its recorded inception in early 1900's (USDA-ERS, 1999). Historically, poultry production was relegated to the small farms

and yards of rural America (USDA-ERS, 1999). As an outgrowth of the egg industry, poultry production existed for the sole traditional purpose of sustenance living and the local retail market. Poultry production was characteristic of small "backyard" flocks of 10-50 chickens and processing of poultry products occurred either close to the source farm or to consumers (USDA-ERS, 1999). In the late 1940's, a new era of poultry production was ushered in with developments in technology, market demand, and policy that lowered poultry production costs to allow for increased profitability (Reimund et. al, 1981). Agricultural research significantly spearheaded the expansion in commercial poultry production with advances in nutrition and disease control, introduction of new breeds, management of poultry environments and products (USDA-ERS, 1999). These major contributors enabled poultry production to be a profitable, productive and viable business venture for the agricultural community (USDA-ERS, 1999). The metamorphosis of the poultry industry led to the abandonment of small-scale poultry operations (flocks of less than 100) and the adoption of large-scale industrial endeavors with flocks of upwards of 500,000 (USDA-ERS, 1999).

Subsequently, rapid growth of the poultry industry and commercial demand has aided in the present-day "conventional" farming of poultry. Conventional farming is defined as standard agricultural practices used widely throughout the U.S. industry that include the use of antibiotics, other antimicrobials and genetically modified organisms (GMOs) in feed. Figure 4 illustrates the conventional practice of poultry production in the United States. A distinctive feature of conventional poultry production in the United States is the organizational scheme. The majority of the U.S. poultry industry operates under a vertically integrated production system (Figure 6) (NCCES, 2007). Vertical integration is a distinct mechanism of shared obligation of production

and processing expenditures via contractual affiliations between farmers and poultry companies (USDA-ERS, 1999). Poultry companies or "integrators" own the processing facilities, hatcheries, and feed mills. Integrators establish production contracts with independent poultry "grow-out" farms for the raising of broilers to market weight (USDA-ERS, 1999). Contract farmers are commonly responsible for providing the land, poultry house(s), and equipment (USDA-ERS, 1999). As well, contractors absorb the costs of labor, utilities, insurance, taxes, waste disposal, and other miscellaneous farm expenses (NCCES, 2007); whereas, the integrator firm generally supplies the feed, bird flocks, medications and supplies. Financial compensation of the contract growers is related to the grower's performance (amount of birds produced) (USDA-ERS, 1999). The arrangement is mutually beneficial: contract farmers avoid large capital investments in feed and birds with less market risk and integrators profit from a constant supply of products with less long-term investment (NCCES, 2007). After World War II, vertical integration progressively became the standard amongst poultry producers in the U.S (USDA-ERS, 1999). By 2003, more than 90 percent of the poultry in the United States was vertically integrated (AgMRC, 2009). Top producers include (in order of production ranking): Tyson Foods, Inc.; Pilgrim's Pride Corp; Gold Kist; Perdue Farms and Sanderson Farms (AgMRC, 2009).

IV. ORGANIC FARMING AND POULTRY

Organic farming has steadily emerged as an important division of agriculture in the United States (AREI, 2006). Organic agriculture is defined as "an ecological production management system that promotes biodiversity, biological cycles and soil health" (NAL, 2007).

Since the adoption of the Organic Foods Production (OFP) Act of 1990, national organic standards have been created for the certification of organic farmland and livestock (NAL, 2007). The extent of organic farming and certified organic farmland quadrupled from 1990 to 2005, and in 2005, all 50 states in the U.S. had certified organic farmland (USDA-ERS, 2002.). Organic acreage of farmland systems are categorized by farm production outputs with cropland and rangeland representing 1.7 and 2.3 million acres, respectively (USDA-ERS, 2002).

Although the practice of organic agriculture is expanding and reaches over 120 countries, obstacles for overall adoption still exist (Morgan & Murdoch, 2000). Certain hindrances to organic adoption practices involve high managerial costs, risks associated with organic transitioning, shortages of organic grains, lack of knowledge regarding organic farming systems, and lack of certified organic processing plants (USDA-ERS, 2002). Nonetheless, the incentive for organic-transition remains with reference to lower input costs, conservation of nonrenewable resources, capitalization on growing niche markets, and ultimate increases in farm income (SARE, 2007).

Within the organic food market, organic meat is the fastest growing sector with growth of over than 67.4 percent to 114 million in 2005 (Figure 5) (NFM, 2006). Organic meat and poultry are considered "gateway" organic products or first organic commodities purchased by a consumer which could dictate future organic purchasing of other products such as cereal or snacks (Dermitt, 2004). Other gateway products include produce, dairy, soy, and baby foods (Dermitt, 2004). Consumer demand for organic meat and poultry have been driven by issues surrounding overuse of antibiotics and growth hormones, the inhumane treatment of livestock, and the natural environment (NBJ, 2004). The organic meat market, as a whole, has been

influenced by the competing "natural" meat industry which is not required to meet USDA organic regulations (USDA-NOP, 2003).

Organic poultry is the largest sector of the organic meat industry (OTA, 2006). Representing 1 percent of the total poultry market, organic poultry production has quadrupled since 2003 with over 13 million of birds in 2005 (Figure 7) (OTA, 2006). Figure 4 depicts a typical organic poultry production facility. California, Pennsylvania, Nebraska and Iowa comprise the top four U.S. states for organic broiler production and accounted for approximately 94 percent of total U.S. organic poultry production in 2005 (Table 3). There is limited knowledge about the structure of the organic poultry industry with some companies being vertically integrated while other poultry companies operate via personal relationships (USDA, 2007). Organic poultry are reared organic from at least day two of life and are at market weight in 70 to 81 days (Dimitre & Greene 2006). The demand for organic poultry has outpaced the supply of organic broilers (AgMRC, 2008). Consumer interest in organic poultry has steadily intensified with more than 7 out of 10 individuals purchasing organic chicken (USDA, 2008). However, hindrances associated with adherence to the OFP Act of 1990 have considerably stifled the short-term industry growth (Greene, 2007). The exceedingly high cost of feed, representing 70% of poultry production expenditures, is a specific obstacle to organic poultry market expansion (Greene, 2007). Yet despite this and other obstacles, organic poultry production is predicted to grow annually upwards of 38% by 2010 (NBJ, 2006).

V. ANTIBIOTIC USAGE AND ANTIBIOTIC RESISTANCE IN AGRICULTURE

The use of antimicrobials in agriculture is a prevalent practice in food animal production. According to Aaerstrup et al. (1999), over 50% of all antimicrobial usage is attributed to food animal production. In the United States and abroad, a wide array of antimicrobial agents are utilized in food animal production (Silbergeld et al., 2008). Table 4 depicts the registered antimicrobials of clinical importance that are used in animal agriculture (FDA, 2004). In food animal production, antimicrobials are administered for therapeutic means for treatment of infection, prophylactic purposes in advance of symptomatic and asymptomatic conditions, and non-therapeutic purposes for growth promotion and improved feed efficiency (Wegener, 2003). The use of growth promoting agents (GPAs) in feed preparations or water supplements illustrate the largest segment of antibiotic use in poultry production (Mellon et al., 2001). In compliance with USDA mandates, GPAs are characterized as "as antibiotics supplements added to the feed of food animals to enhance growth rate and production performance" (Wegener et al., 1999). This differs greatly from antibiotic use for therapeutic and prophylactic purposes which are normally dispensed under higher dosage regimes. To date, a limited body of literature exists substantiating the assertion for improved effects on growth rates, feed conversion efficiencies or general flock quality by way of GPAs (Graham, Boland, & Silbergeld 2007).

An accurate total concerning the amount of antibiotics used for non-therapeutic purposes in animal agriculture remains elusive. The Union of Concerned Scientist conjectures that 24.6 million pounds of antibiotics are utilized for non-therapeutic purposes (Mellon et al., 2001). In contrast, the Animal Health Institute purports that a total of 17.8 million pounds of antibiotics are

used in animal agriculture for the entire spectrum of purposes (AHI, 2000). Overall, the current estimate of non-therapeutic usage of antibiotics in food animal production ranges between 3.1 million pounds to upwards of 25 million pounds in the United States, annually (Mellon et al. , 2001; AHI, 2000). The historic administration of non-therapeutic antibiotics for growth promotion was commercially pioneered in late 1940s and universally adopted within five years (Jukes, 1953). Jukes (1953) contended that the use of a chlortetracycline-amended meal produced faster growing chicks in comparison to soybean-feed counterparts. Mechanisms of growth promotion efficacy are unknown (Visek, 1978). Current dosage of GPAs is prescribed at concentrations below 200 grams per ton of feed for a minimum of 14 days (USDA, 2006).

The usage of GPAs in food animal production is a major public health threat because this practice can contribute to the emergence of antimicrobial resistance worldwide (Levy, 2004; Silbergeld et al., 2008). A myriad of factors contribute to the rise and extent of antimicrobial resistance in both pathogenic and commensal bacteria. Levy et al. (1998) theorizes that the amount and method of antibiotic administration used in food animal production promote the selection of antibiotic-resistant bacteria. Chronic, low-level doses of antibiotics, characteristic of GPAs administered in the animal production environment, encourage the elimination of susceptible bacteria and yield the expansion of resistant bacteria populations (Witte, 2000).

Constitutive and acquired are two forms of resistance to antimicrobial agents (S. Normark & B. Normark, 2002). Constitutive resistance refers to resistance associated with the lack of cellular mechanisms needed for antibiotic susceptibility (S. Normark & B. Normark, 2002). Whereas, acquired resistance denotes genetic-based resistance via chromosomal mutation or the attainment of antibiotic resistance genes via horizontal gene transfer (Prescott, 1999).

Certain significant mechanisms of antimicrobial resistance involve the following: a. enzymatic inactivation of antibiotics, b. failure of antibiotics to permeate through the bacterial cell wall, c. alteration in target receptors, and d. development of enzymes/proteins with low drug affinity (Mazel, 1999).

The clinical importance of bacterial antibiotic resistance is well noted among commensal and pathogenic bacteria in numerous peer-reviewed studies. Barza et al. (2002) estimated that an attributable fraction of between 13% and 26% of drug-resistant *Salmonella* infections are acquired through an antibiotic resistance. Many drugs used in veterinary medicine have identical analogs that are used in human medicine (Khachatourians, 1998; Smith, 2005). Animal-derived antibiotic-resistant bacteria can colonize the intestinal flora of humans. Donabedian et al. (2003) provided molecular evidence of animal –human transfer of gentamicin resistance in *Enterococcus* isolates through food. To address concerns associated with antimicrobial resistance, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 to survey antibiotic-resistant bacteria in humans, retail meats and the agricultural environment (NARMS, 2009). Ultimately, extensive and improper use of antibiotic drugs in food animal agriculture can establish reservoirs of antibiotic-resistant bacteria, greatly impacting public health (Levy, 2004; van den Bogaard, 2000).

Chapter 3

Methodology

Chapter 3: Methodology

I. STUDY SITE DESCRIPTION

Two types of farms were included in this study: large-scale conventional poultry farms that housed >15,000 broilers per house (control farms), and large-scale conventional poultry farms that were within the first year of transitioning to organic practices (intervention farms). Characteristic differences between the conventional and organic-transitioning poultry farms (see Glossary) included size, birds/house, amount of sunlight, and antibiotic and chemical usage. All farms were located in the Mid Atlantic region of the U.S.

II. SAMPLE COLLECTION

From March 2008- June 2008, environmental samples were collected from control poultry houses (n=10) and intervention poultry houses (n=10). Three main types of samples were collected from each house: poultry litter, water, and feed.

Three 500g poultry litter samples (~500g) from the top 1 to 2 cm of litter were aseptically collected from 3 locations defined by a 0.5-1.0 m^2 area within each poultry house. One sample was collected in the middle of the house away from automated feed and water lines, one sample was collected from beneath automated feed lines and one sample was collected from beneath automated water lines. Air flow, water activity (Aw) and ambient light also were measured at each poultry litter sampling location. Air flow and ambient light were measured using a light meter, respectively, six inches above each litter sampling location and Aw was measured using a calibrated water activity meter (PawKit, New York, NY).

Two water samples (~600 mL) were retrieved using sterile Whirl-Pak® collection bags (Nasco, Fort Atkinson, WI) from raw source water (**before** any possible UV or chlorination treatment) and finished water (water provided to broilers **after** any possible UV or chlorination treatment) from each poultry house. One poultry feed sample (~ 300 g) was collected in a sterile Whirl-Pak® collection bag (Nasco, Fort Atkinson, WI) from the feed hopper within each house. All poultry litter, water and feed samples were mailed overnight and processed in the laboratory for the cultivation and isolation of *Enterococcus* spp. and *Salmonella* spp. within 24 hours.

III. POULTRY FARM QUESTIONIARRE: Environmental Indices

To elucidate the influence of environmental factors on prevalence of susceptible and antibiotic-resistant bacteria at all participating farms, a study questionnaire was developed (See Appendix). Data concerning ambient conditions were collected by including questions about the date, season, ambient air temperature inside and outside of poultry houses, relative humidity inside and outside of poultry houses and rainfall. Breeder practice data was collected by including questions regarding the types of breeder birds, breeder company, and antibiotic usage on breeder farm. Hatchery practices variables incorporated information involving hatchery company name and antibiotic usage at hatchery. In reference to grower farm characteristics, examined environmental variables included the following: grower company, number of weeks since transition to organic practices began, geographic locations, distance to other conventional or organic poultry farms, poultry house size, type of ventilation system in poultry house, air flow in poultry house; square footage allowance per bird, average time spent outside by flock per day,

amount of sunlight in poultry house, type of water in nipple feeders, type of feed, type of poultry litter, poultry litter management practices, poultry litter water activity. Lastly, bird characteristics were also documented and utilized in the integration of environmental factor analysis.

IV. ISOLATION

A. Isolation and Enumeration of Enterococcus spp. and Salmonella spp. from Water

Isolation of *Enterococcus* spp. and *Salmonella* spp. from water samples was performed in accordance with standard membrane filtration methods: U.S. Environmental Protection Agency (EPA) Method 1106.1 and Method 1103 (U.S EPA, 2000), and standard method SM 9222D [American Public Health Association APHA 1998]. Dilutions of each water sample (10^0, 10^{-1}, 10^{-2}, and 10^{-3}) were prepared, and 10 mL of each dilution, as well as 10 mL and 100 mL of each original sample, was filtered through 0.45um (cut size), 47 mm (diameter size) mixed cellulose ester filters (Millipore, Billerica, MA). Each filter was placed on *Enterococcus* Agar (EA) and XLT4 Agar for the isolation of *Enterococcus* spp. and *Salmonella* spp., respectively. Throughout the water membrane filtration method, negative control filters were employed for quality control and assurance. All water sample filters were incubated at 41°C for *Enterococcus* and 37°C for *Salmonella* for 24 hr. Presumptive colonies of *Enterococcus* ranged in appearance from brown to black with a brown-black precipitate on EA agar. Similarly, colony morphology for presumptive *Salmonella* spp. was indicative of black colonies associated with a yellow color change on XLT4 agar. Enumeration of resulting colonies and concentrations of *Enterococcus*

spp. and *Salmonella* spp. per 100 mL water were ascertained using back calculations from dilution plates containing 30-300 CFU. Of recovered presumptive *Enterococcus* spp. and *Salmonella* spp., three bacterial isolates per water sample were archived in Brucella broth with 20% glycerol at -80°C.

B. *Isolation of Enterococcus spp. From Poultry Litter and Poultry Feed Samples*

Poultry litter and feed samples were enriched in a 1:10 weight to volume dilution of 100 mL of Enterococcosel Broth for 24 hr at 41°C. Positive and negative control broths were included in this experiment for quality control and assurance. After 24 hr, 10 uL of the enrichment culture was streaked onto Enterococcosel Agar (EA) and incubated overnight at 41°C. A single positive colony was streaked onto Brain Heart Infusion (BHI) agar, a non-selective media, for purification of presumptive *Enterococcus* isolates and incubated at 41°C for 24 hr. A substantial colony swab was collected from each BHI agar purification plate and archived at -80 °C in Brucella broth with 20% glycerol.

C. *Isolation of Salmonella spp. From Poultry Litter and Feed Samples*

Salmonella spp. were recovered from poultry litter and feed samples using a two-step enrichment process. Initially, litter and feed samples were pre-enriched in a 1:10 weight to volume dilution of 100 mL of Lactose Broth for 24 hr at 37°C. From the Lactose Broth suspension, an aliquot (1mL) of the suspension was added to 15 mL of Hajna Tetrathionate Broth supplemented with a prepared iodine solution (1.2 mL per 15mL of Hajna) and incubated

overnight at 37°C. Control (positive and negative) broths and agar plates were included for quality control and assurance. After 24 hr, 10 uL of enrichment culture was streaked onto XLT4 Agar and incubated at 37°C overnight for the isolation of *Salmonella* spp. For samples that were initially *Salmonella*-negative using this method, a secondary enrichment-recovery was executed which entailed leaving the TT Hajna enrichment on the bench top for an additional 4-5 days and subsequently streaking a loopful of the suspensions onto XLT4 agar plates. After which, a single positive colony was streaked onto Brain Heart Infusion (BHI) agar, a non-selective media, for purification of presumptive *Salmonella* isolates and incubated at 37°C for 24 hr. A generous swab of colonies was collected from each BHI agar purification plate and archived at -80 °C in Brucella broth with 20% glycerol.

V. IDENTIFICATION

A. Identification of Enterococcus Recovered from Water, Poultry Litter and Poultry Feed Samples

Briefly, all presumptive *Enterococcus* isolates were streaked from archival stocks onto Blood Agar Plates and incubated at 41°C for 24 hr. For presumptive identification of *Enterococcus* spp. from water, poultry litter and feed samples, a biochemical screening process (in order of method) was employed: gram staining for appearance of gram-positive cocci; catalase test for the production of catalase in the presence of 3% hydrogen peroxide; and PYR testing for the enzymatic activity of pyrolidonyl-arylamidase (PYRase). All gram-positive, catalase negative, and PYR test positive isolates were confirmed and identified to the species-level using the automated biochemical identification Vitek ® System (Vitek ®Compact 2;

BioMérieux Vitek Systems Inc., Hazelwood, MO) in accordance with the manufacturer's specifications. Vitek 2 Compact Gram-Positive (GP) colorimetric cards were utilized for the interpretation of a suite of biochemical screening tests appropriate for *Enterococcus* spp.

B. Identification of Salmonella Recovered from Water, Poultry Litter and Poultry Feed Samples

Briefly, all presumptive *Salmonella* isolates were streaked from archival stocks onto Blood Agar Plates and incubated at 37°C for 24 hr. The biochemical screening tests performed on presumptive *Salmonella* spp. recovered from poultry litter, water and feed samples included (in order of method) Gram Staining, the oxidase test, the Lysine Iron Agar (LIA) test, and the Triple Sugar Iron Agar (TSI) test. All gram-negative, oxidase positive, LIA positive (alkaline slant: alkaline butt) and TSI positive (alkaline slant: acid butt) isolates as described by the FDA Bacteriological Analytical Manual (http://www.cfsan.fda.gov/~ebam/bam-5.html#Id) were presumptively identified as *Salmonella*. Positive cultures were confirmed using the automated biochemical identification Vitek ®System (Vitek ®Compact 2; bioMérieux Vitek Systems Inc., Hazelwood, MO) by the manufacturer's guidance. Vitek 2 Compact Gram-Negative (GN) colorimetric cards were utilized for the interpretation of a suite of biochemical screening tests appropriate for genus-level identification of *Salmonella spp.* For speciation, *Salmonella spp*. were identified utilizing serological methods described in the FDA Center for Veterinary Medicine Food and Animal Microbiology Laboratory Standard Operating Procedures (530-058 R-1): *Serology of Salmonella Spp. Isolates* using CDC antisera and Difco antisera (Becton Dickinson Microbiology System, Cockeysville, MD, USA).

VI. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed on all *Enterococcus* (n=394) and *Salmonella* (n=121) isolates. As depicted in Figure 1, antimicrobial minimal inhibitory concentration (MIC) values for *Enterococcus and Salmonella* isolates were determined using the automated Sensititre™ antimicrobial susceptibility testing system (Trek Diagnostic Systems, Westlake, Ohio) according to the manufacturer's directions. A 0.5 McFarland Standard was placed into a nephelometer for calibration. Colonies from pure 18-24hr cultures were transferred to sterile Sensititre demineralized water. 50 μL of suspension (*Enterococcus*) and 30 μL of suspension (*Salmonella*) were added to sterile of Sensititre cation-adjusted Mueller Hinton broth. Briefly, an approximate 5 X 10^5 CFU/mL inoculum of each isolate was prepared from a Mueller-Hinton broth suspension to achieve a turbidity equivalent to a 0.5 McFarland standard. The final inoculate (50uL) was dispensed into microtitre 96-well plates embedded with test antimicrobials. Plates were incubated in the Automated Reading and Incubation System (ARIS) at 37°C for 18 ± 1hr. The first 100 plates were read both manually and via the ARIS system for quality assurance comparisons of MIC determinations; subsequent samples were read by ARIS exclusively.

Sensititre susceptibility testing was performed with the following antibiotics: (*Enterococcus spp*.) chloramphenicol, ciprofloxacin, daptomycin, erythromycin, flavomycin, gentamicin, kanamycin, lincomycin, linezolid, nitrofurantoin, penicillin, streptomycin, quinupristin/dalfopristin, tetracycline, tigecycline, tylosin, and vancomycin; and *(Salmonella*

spp.*)* amikacin, augmentin, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines for broth- microdilution methods (www.clsi.org;M31-A3), interpretation criteria were used to evaluate resulting MICs. *Enterococcus faecalis* ATCC 29212 and ATCC 51299, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains in antimicrobial MIC determinations. Inconclusive and indefinable ARIS system readings for MIC results were repeated.

VII. STATISTICAL ANALYSIS

Enterococcus spp. (n=260) and *Salmonella* spp. (n=121) recovered from all positive poultry farm samples were included in the statistical analysis. General linear latent and mixed models (GLLAMM) were used to evaluate associations between rates of antibiotic-resistant *Enterococcus* spp. and *Salmonella* spp. and on-farm environmental factors, including poultry production type (i.e. conventional or organic-transitioning). The GLLAMM method was used to account for the clustered nature of the study design which made it necessary to adjust for intra-poultry house and intra-poultry farm variability. Within the GLLAMM framework, environmental factors were individually modeled against the suite antibiotic resistance data for *Enterococcus* spp. (gram-positive) and *Salmonella* spp. (gram-negative). A step-wise construction of antibiotic-specific models incorporating multiple significant environmental variables was employed yielding consideration to potential collinearity between variables. Subsequently, odds ratio were determined to compare the odds of antibiotic resistance between

production types integrating selected environmental factors. All statistical analyses were performed using STATA10 (Stata Corporation, College Station, TX) and in all cases, p values $\leq$ 0.05 were regarded as statistically significant.

Chapter 4

Results

Chapter 4: Results

I. POULTRY HOUSE CHARACTERISTICS

Table 5 summarizes descriptive data concerning poultry house characteristics, meteorological conditions, and broiler information surveyed in organic-transitioning (n=10) and conventional (n=10) poultry operations in the Mid-Atlantic United States from March 31, 2008 to June 2, 2008. Organic-transitioning poultry houses, on average, were characterized by a seven-year house age difference compared with conventional counterparts. At the time of sampling, average duration of organic-transitioning practices in poultry houses was 1.7 months. Comparatively, conventional poultry houses exhibited higher mean values for several meteorological and environmental parameters including inside temperature, inside humidity, and water activity. Conventional poultry houses were characterized by an average of 8,250 additional broilers/house, and broilers that were an average of 0.3 weeks older than organic-transitioning poultry house equivalents. Organic-transitioning poultry houses demonstrated structural differences from conventional houses. Average length, width and ambient light of organic-transitioning poultry houses were greater than that of the conventional group. The mean mortality rate was 4.7% in organic poultry houses compared to 2.56% in conventional houses. Neither organic-transitioning nor conventional poultry houses documented time outdoors for broilers at the time of baseline sampling.

II. *ENTEROCOCCUS*

A. Prevalence of Enterococcus spp.

Enterococcus spp. were isolated from every organic-transitioning and conventional poultry farm tested during this baseline sampling period. In environmental samples recovered from conventional and organic poultry production house environments, the prevalence of *Enterococcus* spp. was 100%. The prevalence of *Enterococcus* species was prominent across all environmental media. Interestingly, 60 percent of the waterline samples in conventional poultry houses were *Enterococcus* spp. positive compared to 30% in organic-transitioning settings. In this study, the predominant environmental media for the recovery of *Enterococcus* spp. isolates between both production types was poultry litter with similar distribution across litter sample location (Table 6).

The predominant *Enterococcus* species from all environmental samples, constituting approximately 50.4% of total isolated *Enterococcus* spp., was *E. faecium* (Table 7). Within each type of production practice, the most common species isolated was *E. faecalis* (50%) in organic-transitioning and *E. faecium* (52%) in conventional production (Table 7). Organic-transitioning poultry houses reveal greater species diversity among *Enterococcus* spp. (Table 7). As presented in Table 8, a broad distribution of *Enterococcus* species was evident in a variety of environmental media sampled. In conventional settings, E. *faecium* was the predominant species isolated from every environmental media sample except poultry source water. Ninety-three percent of poultry waterline isolates from conventional poultry houses were *E. faecium* (Table 8). Conventional poultry houses demonstrated greater *Enterococcus* species diversity within

poultry feed samples. With respect to organic-transitioning production environments, E. *faecalis* was isolated solely from within poultry litter samples (Table 8). Of the total environmental isolates, the non-*Enterococcus* spp. recovered were the following: thirty-two (11%) isolates were identified as *Staphylococcus* spp., 3 (1%) isolates were *Pedioccocus spp*. and 4 (1.35%) isolates were unidentified.

B. Antibiotic Resistance of Enterococcus spp.: Minimal inhibitory concentration (MIC) distributions

Classified by production type, the MIC distributions of *Enterococcus* spp. are summarized in Table 9. Overall, all *Enterococcus* spp. isolates from conventional and organic-transitioning poultry houses displayed similar ranges of MICs. Five (36%) antimicrobial agents associated with conventional settings had wider MIC ranges than organic-transitioning *Enterococcus* isolates. Conventional poultry houses were characterized by a greater number of isolates expressing the highest MICs. Inter-species differences were evident among *E. faecalis* and *E. faecium* MICs associated with the different types of poultry houses (Table 10 & Table 11). Conventional *E. faecium* isolates expressed a wider range of MICs and a greater number of isolates at the uppermost MIC compared to *E. faecalis* isolates. For example, conventional *E. faecium* isolates had wider MIC ranges for 9 (66%) antimicrobial agents compared to that of organic-transitioning isolates.

C. Antibiotic Resistance of Enterococcus spp.: Resistance Patterns

Conventional *Enterococcus* spp. isolates expressed resistance to a higher number of antibiotics compared to organic-transitioning isolates (Figure 8). In general, intermediate resistance to a greater number of antibiotics was more common among organic-transitioning *Enterococcus* isolates as shown in Figure 9. Conventional *Enterococcus* isolates displayed overall higher percentages of resistance to across 83% (14/17) of antibiotic agents compared to organic isolates (Figure 10).

Statistically significant differences were evident between production types for 9 out of 14 antibiotics tested with isolates recovered from conventional poultry houses expressing elevated percent resistance compared to organic poultry houses (Table 17)

None of the organic-transitioning *Enterococcus* isolates expressed resistance to chloramphenicol, gentamicin, linezolid, and tigecycline. In terms of both organic-transitioning and conventional poultry houses, all isolates displayed susceptibility to daptomycin, flavomycin, and vancomycin. Similar antibiotic resistance patterns were evident for erythromycin and tylosin, which are both constituents of the macrolide antibiotic class. Within poultry production type, several *Enterococcus* spp. expressed multi-drug resistance. Over a third of *Enterococcus* isolated from conventional poultry environments (37%) were resistant to at least three antimicrobial classes compared to 10.3% of *Enterococcus* isolates in organic-transitioning settings in this study. Relative to organic-transitioning poultry houses, *E. faecium* isolates from conventional houses expressed elevated levels of percent antibiotic resistance (Figure 11). The most prominent differences occurred with penicillin (2.38% vs. 52.86%) ($p \leq 0.05$) and

tetracycline (11.90% vs. 81.43%) ($p \leq 0.05$). As illustrated in Figure 12, *E. faecalis* isolates from conventional broiler production expressed higher resistance to the majority of antibiotics tested except for tetracycline. Due to intrinsic resistance, both organic-transitioning and conventional *E. faecalis* isolates expressed complete antibiotic resistance to lincomycin and similar rates of resistance for Synercid, a human analog of virginiamycin. In addition, the *Pediococcus* spp. (n=3) isolated from feed samples were resistant to vancomycin, an intrinsic trait of that species.

III. SALMONELLA.

A. Prevalence of Salmonella spp.

During this baseline sampling period, the prevalence of *Salmonella* spp. on organic-transitioning and conventional poultry farms in this study was 100% and 40%, respectively. *Salmonella* spp. were isolated from several organic-transitioning (n=8) and conventional poultry houses (n=3). The prevalence of Salmonella within organic poultry houses in this study was 80% compared to 30% in conventional poultry houses. In this study, the predominant environmental media for the recovery of *Salmonella* spp. isolates was poultry litter 2 (under waterline) in organic-transitioning poultry houses and poultry feed in conventional poultry houses (Table 11).

An array of *Salmonella* serovars were identified from both conventional and organic-transitioning poultry houses (Table 12). *S. kentucky* was the predominant serovar; accounting for 63% of total *Salmonella* spp. isolates (Table 13). *Salmonella* spp. were present in three

types of environmental media (poultry litter, feed and soil) recovered from conventional poultry houses; however, *Salmonella* was only found in poultry litter collected from organic-transitioning poultry houses (Table 14). Of conventional poultry houses isolates, *S. kentucky* was exclusively isolated from poultry litter. Of the total environmental isolates, the non-*Salmonella* spp. recovered were the following: three (2.7%) isolates were identified as *Escherichia coli e coli*, 1 (.9%) isolate was lactose-fermenting *E. coli*, 3 (2.7%) were *Pseudomonas luteola isolates*, and 3(2.7%) isolates were *Citrobacter freundi*.

B. Antibiotic Resistance of Salmonella spp.: MIC Distributions

MIC distributions of *Salmonella* spp. categorized by production type are presented in Table (Table 15). On the whole, organic-transitioning poultry houses consistently displayed a higher number of isolates within the lower concentration range of the MIC distribution for all antibiotics tested. Conventional poultry houses displayed a higher number of isolates within the upper concentration range of the MIC distribution for augmentin, ampicillin, and ceftriaxone. Consistently, conventional and organic-transitioning poultry houses displayed analogous ranges of MICs for all *Salmonella* spp. isolates. Five (33%) antimicrobial agents associated with organic-transitioning production environments had wider MIC ranges for *Salmonella* isolates than those recovered from conventional settings.

C. Antibiotic Resistance of Salmonella spp.: Resistance Patterns

Conventional and organic-transitioning *Salmonella* spp. isolates exhibited resistance to a similar suite of antibiotics. (Figure 13). Intermediate resistance to a higher number of

antibiotics was more common among organic-transitioning *Salmonella* spp. isolates (Figure 14). Conventional *Salmonella* spp. isolates displayed a higher percent resistance compared to organic isolates for augmentin, ampicillin, cefoxitin, and ceftiofur (Figure 15). Among the aforementioned antibiotics, there were no significant differences between production types. Similar antibiotic resistance patterns were observed for streptomycin and tetracycline, members of the aminoglycoside and tetracycline antibiotic classes, respectively. In addition, there were no significant differences in resistance to these antibiotics between isolates from conventional and organic-transitioning poultry houses (Table 15). None of the *Salmonella* spp. isolates expressed resistance to amikacin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulfisoxazole, and trimethoprim/sulfamethoxazone antimicrobial agents. Within both production types, only *S. kentucky* isolates expressed resistance to any particular antibiotic with remaining serovars being susceptible to all antimicrobials tested (Figure 16). Multi-drug-resistant *Salmonella spp.* isolates also were prevalent in this baseline study (Table 16). Sixty-two percent of organic-transitioning S. kentucky isolates were resistant to at least 2 antimicrobials, and 21% of conventional *S. Kentucky* were resistant to at least 2 antimicrobials. Of highlight, 21% of 24 conventional *S. kentucky* isolates were resistant to 6 antimicrobials tested compared to 4% of organic isolates.

IV. ENVIRONMENTAL FACTORS

Percent resistance to all antibiotics was compared between conventional and organic-transitioning houses using the GLLAMM model. Several environmental variables were also modeled using GLLAMM to determine whether associations existed between environmental

factors within the poultry production environment and the prevalence of antibiotic-resistant *Enterococcus* spp. and *Salmonella* spp..

A. *Enterococcus*

Enterococcus spp. p-values were compared in association with individual environmental variables in conventional and organic-transitioning poultry house adjusting for intra-poultry house and farm variation modeled across the suite of tested antibiotics. In tandem, collinearity was examined between selected environmental factors prior to incorporation into antibiotic-specific multivariate model using GLLAMM with highly collinear variables (>0.5) excised from the model (Table 17). Only streptomycin (STR) resistance was statistically significantly different between conventional and organic-transitioning poultry houses in association with any environmental factors modeled using GLLAMM when adjusted for intra-poultry house and-farm variation. Humidity inside of the poultry houses was successfully model fit with significant STR resistance differences between production systems. In our study, there is a 2.7 times more likely odds of STR resistant *Enterococcus* isolates recovered from conventional poultry houses compared to organic-transitioning poultry houses (95% CI =[1.2 ,6.0]; P<0.017), after controlling for humidity inside the house. In addition for every unit increase in humidity the odds of STR resistance in *Enterococcus* isolates within conventional poultry houses decreases by a factor of .96 compared to organic-transitioning poultry houses (95% CI =[.93,.99]; P<0.011) .

B. *Salmonella*

There was no statistically significant difference in expressed resistance to a particular antibiotic between conventional and organic-transitioning poultry houses across the entire suite of tested antibiotics. Therefore, analysis of the influence of environmental variables and odds ratio calculations were not ascertained.

.

Chapter 5

Discussion and Conclusions

Chapter 5: Discussion and Conclusions

I. DISCUSSION

In this baseline study, antibiotic-resistant *Enterococcus* and *Salmonella* were isolated from conventional and organic-transitioning poultry farms. This is the first epidemiological on-farm study in United States to investigate temporal trends in antibiotic resistance of *Salmonella* spp. and *Enterococcus* spp. recovered from poultry farms undergoing conversion to organic practices and discontinuing the use of GPAs. Moreover, this study is novel in the examination of potential environmental risk factors for antibiotic resistance in conventional and organic-transitioning poultry farms.

Overall, results confirm differences in prevalence and antimicrobial susceptibility of *Enterococcus* and *Salmonella* spp. recovered from conventional versus organic-transitioning poultry production systems. Specific findings indicate that there is an elevated prevalence of *Enterococcus* spp. in samples recovered from conventional poultry production environments and a greater percentage of these isolates are resistant to a suite of antibiotics compared to isolates recovered from organic-transitioning poultry environments (Table and Figure 10). As depicted in Table 12, there was a higher prevalence of *Salmonella* isolates recovered from organic-transitioning poultry houses during time of sampling. Two major antibiotic resistant patterns emerged from examination of *Salmonella* isolates obtained from differing broiler production systems (Figure 15). Members of the β-lactams and cephems antimicrobial class exhibited higher percentage of resistance in organic-transitioning poultry houses than conventional poultry

house complements(Table and Figure 15). There were no *Enterococcus* isolates resistant to daptomycin, flavomycin or vancomycin. This result makes sense because vancomycin was never approved for use in poultry production in the United States (USDA, 2001). In addition, there were no statistically significant differences in antibiotic resistance between production types for older generation antibiotics such as lincomycin (with regard to *Enterococcus* isolates) and streptomycin and tetracycline (with regard to *Salmonella* isolates).

Because this was a baseline study, our purpose was to examine preliminary prevalence and antibiotic resistance of *Enterococcus* spp. and *Salmonella* spp. by production type within the first-year of the study. As the foundation for a 4-year long-term study, our investigation did not anticipate significant differences for a majority of antibiotics tested due in part to the initial stage of the study. In capturing the most accurate measure of the epidemiological intervention on production practices within poultry environments, study sampling commenced at the earliest point of conversion. The lion's share of organic-transitioning farms (the intervention group) had only recently undergone conversion--on average, they had converted within the previous 2 months before initial sampling. Intuitively, it is most probable that the organic-transitioning poultry houses more mirrored conventional counterparts and thus significant differences between production types would not be as apparent at time of sampling. This is a viable justification for the low prevalence and absence of significant resistance pattern differences between production types for *Enterococcus* spp. and, in particular the majority of *Salmonella* spp., isolated in this study. To this end, an explanation for the lack of associations between environmental factors and antibiotic resistance can also be explained by this phenomenon.

A. Enterococcus

In environmental samples collected from conventional and organic-transitioning poultry farms, the prevalence of *Enterococcus* was 100%. *Enterococcus faecium* proved to be a pervasive organism across the majority of environmental media. The results indicate higher levels species diversity observed in organic-transitioning poultry houses. As the organic-transitioning poultry houses do not utilize antibiotics across any aspect of the production continuum, it is probable that a more diverse population of *Enterococcus* spp. could be established due to the absence of selection pressures associated with antibiotics which can normally displace susceptible *Enterococcus* species. The results indicate a statistically significant difference between poultry production systems for *Enterococcus* resistance to a majority of the antibiotics tested in the direction of conventional poultry production. These findings are in agreement with cross-sectional studies comparing conventional and a variety of poultry production practices along the organic certification spectrum where data reveals higher percentages of antibiotic-resistant bacteria on conventional poultry farms and conventional poultry products. In a one-year cross-sectional study of conventional and organic poultry houses, findings from Joseph et al, 2006 indicated that the percentage of *E. faecium* resistant to nine different classes of antibiotics was statistically significantly higher among isolates recovered from conventional poultry houses compared to organic poultry houses. More specifically, 98% of *E. faecium* from conventional poultry houses were resistant to erythromycin, compared to 43% of isolates recovered from organic poultry houses (OR=50.9, 95% CI = 22.1-134.4) (Joseph et al. 2006). Similarly a study conducted by Price et al (2005) which compared fluoroquinolone-resistant *Campylobacter* spp. on conventional versus antibiotic-free poultry

products found conventional products had statistically significantly higher odds of carrying resistant isolates compared to antibiotic-free products.

B. Salmonella

There was a more striking contrast in *Salmonella* prevalence between samples originating from conventional versus organic-transitioning poultry farms, with samples from organic-transitioning farms characterized by an approximately 2.5-fold higher prevalence of *Salmonella* spp. These findings are generally consistent with previous studies that have observed higher loads of *Salmonella* in organic poultry farms and products compared to conventional counterparts (Cui et al., 2005; Miranda et al., 2007; Van et al., 2006). An explanation for increased prevalence of *Salmonella* in organic settings has been hypothesized to involve contact with potential environmental reservoirs of *Salmonella* infections. As mandated with the organic certification process for poultry production, organic farms must afford poultry flock access to the outside environment. Piekus (2008) theorizes, outdoor access may increase the risk of *Salmonella* infection via contact with feces of wild birds or other animals near organic poultry production facilities. Some of the major animal reservoirs include cats, dogs, opossums, rodents, raccoons, badgers, chipmunks, and skunks via fecal shedding (Jacob, Griggs, & Bender 2008). Specifically, mice represent a perpetual source of *Salmonella*, principally, S. *enteritidis* (Davies & Wray, 1996). However, in our study poultry growers reported that the organic-transitioning broilers never roamed outside even when provided access to an outdoor area; indicating that biological vectors presumably did not play a role in *Salmonella* prevalence in organic-transitioning poultry houses.

The predominant serovar of *Salmonella* found in both types of production environments was *S. Kentucky* which was also the only serovar to express resistance to a particular antibiotic. Interestingly, feed contamination via *Salmonella* species was prevalent in conventional poultry houses in this study, exclusively. Historically, there has been directed inference that formulated feeds are principal contributors in the introduction of resistant food-borne bacteria (Levy, 1998) in agricultural settings. However, the organic-transitioning poultry houses which discontinued use of GAPs, expressed antibiotic-resistance for several *Salmonella* spp. isolates in this study. This study further provides evidence in which other environmental inputs could be potentially contributing to the prevalence and antibiotic resistance of *Salmonella* spp. in poultry production environments.

Production type played no significant role in percentages of antibiotic-resistant *Salmonella*. Similar levels of antibiotic resistance in both organic and conventional poultry facilities have been observed in other gram-negative bacteria species. Luangtongkum et al. (2006) also observed high levels of tetracycline-resistant *Campylobacter* spp. among isolates from both organic and conventional poultry farms. Upon reflection on the preponderance of *Salmonella* multi-drug resistance in conventional poultry settings, our baseline data suggests differences in the expression of multi-drug resistance profiles based on production practices. These findings support evidence from Cui et al. (2005) which reported that 100% of *Salmonella enterica* serovar Typhimurium isolates recovered from conventional poultry products purchased in Maryland grocery stores were resistant to at least 5 antimicrobials, while 79% of isolates recovered from organic poultry products were susceptible to 17 antimicrobials. Likewise, Ray et al. (2006) observed that conventional dairy farms tend to be more likely to have at least one

Salmonella isolate resistant to five or more antimicrobial agents when compared with organic farms. However, the small number of *Salmonella* spp. in this study does not permit a conclusive statement.

C. Environmental Factors on Antibiotic Resistance in Poultry Houses

A variety of environmental factors were examined to evaluate whether they impacted the prevalence of antibiotic-resistant bacteria in conventional and organic-transitioning poultry houses. Inside humidity was statistically significantly associated with differences in prevalence of STR-resistant *Enterococcus* spp. between conventional and organic-transitioning poultry houses. Humidity provides a suitable environment for bacterial growth in general, may contribute to the spreading of bacterial contamination throughout poultry houses Coupled with the inherent genetic promiscuity of *Enterococcus* spp., elevated bacterial population levels may yield the opportunity for potential acquisition and proliferation of resident antibiotic resistant gene elements throughout the poultry production environment. However, a biologically plausible explanation for an association between humidity and the decrease in antibiotic resistance of Streptomycin can be ascribed to thermal stress. A threshold response to thermal stress associated with increased humidity could contribute to reversion of resistant bacteria to susceptible wild-type over considerable time. Further research is warranted to investigate a more conclusive interpretation.

At the time of sampling, statistically significant differences in antibiotic resistance were not evident for *Salmonella* spp. between conventional and organic-transitioning poultry houses.

This finding is not surprising due to the time of sampling and the baseline stage of the longitudinal study. Sampling was performed at the beginning of flock introduction within the poultry houses which may impact the dynamics of antibiotic resistance and influence of environmental factors on resistance. As to provide the most accurate baseline data, the organic-transitioning poultry houses were chosen at the genesis of their conversion to organic practices. Poultry houses in the organic-transitioning (intervention) group were, on average, 3 months removed from the practices associated with conventional poultry farms. Therefore, significant differences between production types based on the adoption of organic practices would be seemingly premature and inconsistent with previous studies. Several studies have documented differences in antibiotic resistance and prevalence of bacteria over a more extended time-span. For example, Aarestrup (2001) revealed a 14-fold decrease in the prevalence of glycopeptide-resistant *E .faecium* in broilers following a 1995 Danish avoparcin ban, after a five-year time period (72.7% in 1995 to 5.8% in 2000).

II. LIMITATIONS

This baseline study had several major limitations which are inherent in epidemiological research and artifacts of preliminary data collection. Firstly, the study is limited by geographical location in that all poultry farms were located in the Mid-Atlantic United States. However, poultry products produced in this area are widely distributed in the United States; therefore, the limited geographical locations covered in this study would not likely affect the generalizability of the results. Secondly, recovery of *Salmonella* across all environmental samples was potentially limited by our isolation techniques. Some studies have shown that a Rappaport-

Vassiliadis (RV) enrichment protocol may be more effective in isolating *Salmonella*, i.e. water samples, compared to TT-Hajna enrichment protocol that we used. This may have been why we isolated a relatively low number of *Salmonella* compared to *Enterococcus.* In addition, we were not able to capture on-farm levels of pH in environmental media which may play a significant role in the prevalence of bacterial organisms since pH is a limiting factor for bacterial growth. Thirdly, the short sampling period proved to be a hindrance in analyzing environmental associations over time, including the potential effect of seasonal variation. Seasonal variation will be adjusted for with subsequent sampling seasons during the ensuing 4-year study. Lastly, sample independence was an issue in this study because samples were clustered due to the collection of multiple environmental samples within the same poultry house and the same poultry farm. The selection of the GLLAMM for binary outcomes was chosen to adjust for intra-poultry house and intra-poultry farm variation between sample isolates recovered in the study.

III. PUBLIC HEALTH IMPLICATIONS

Within the industrial farm animal production complex, the use of GPAs in food-animal production could present a potential human health concern with regard to exposures to antibiotic-resistant bacteria and; thereby, significantly compromise the efficacy of the arsenal of antibiotics utilized for treatment of clinical infections in the United States. By way of a recent declaration by the Infectious Disease Society of America (ISDA),infections associated with antibiotic resistance have been deemed as a public health “epidemic” in the United States (Spellberg et al., 2008). Direct epidemiological analyses relay that antimicrobial-resistant bacteria populations

derived from food animals can be transmitted to human populations (van den Bogaard, 2000; Angulo, Nagrund & Chiller, 2004). Presently, the emergence and persistence of antibiotic resistance in pathogenic and commensal food-borne bacteria, *including Campylobacter spp*., *Salmonella* spp. and *Enterococcus* spp. endangers individual human health, as well as the public health infrastructure (Altekruse, 1999; Heuer, 2006; IOM, 1998; Molbak, 2005). Conservative estimates suggest that the attributable fraction of food-borne antibiotic- resistant non-typhoidal *Salmonella* spp. and. infections from food animals is 2.6%, (Barza, 2002). Specifically, there is evidence that the use of antimicrobials, specifically GPAs, in poultry production could be a contributor to the development of antibiotic resistance in pathogenic and commensal food-borne bacteria (Gorbach, 2001; Idris, 2006; NRC, 1999; Wegener, 2003). Internationally, a number of studies have explored the role of poultry production in the rise of antibiotic-resistant bacteria (Wegener, 1999; Heuer, 2001; Bywater, 2004). EU countries, in response to this potential public health threat, banned four growth promoters (bacitracin, tylosin, spiramycin, and virginiamycin) in 1998 due to structure and mechanistic relatedness to human antibiotic equivalents (EU Commission, 2003).

From a public-health perspective, our specific study demonstrates the effectiveness of an intervention initiative promoting the cessation of antibiotic use within poultry production environments. Organic-transitioning, as characterized by the discontinuation of antibiotic use, may lead to significant reductions in antibiotic-resistance in poultry environment over time. We observed nascent reductions in antibiotic resistance of selected food-borne bacteria in association with production practice conversion. Subsequently, the alteration of production operations may lead to lower risks associated with exposure to resistant food-borne bacteria either directly

(contact with food-animals) or indirectly (consumption of foodstuffs contaminated with resistant food-borne bacteria) in connection to poultry production. This baseline study generates a primary scientific source for a U.S. scenario involving the complete abolishment of antibiotics of public health importance in food animal production. The outcome of this study could prove very timely and influential within the national political landscape. On March 17, 2009, Congresswoman Louise M. Slaughter and Senator Edward Kennedy introduced a bill to curtail excessive usage of antibiotics in the Nation's food supply by advocating the phase out of antibiotic formulations utilized in both human and veterinary medicine in food animal production. The legislation, Preservation of Antibiotics for Medical Treatment Act (H.R. 1549/S. 619), would be enacted to:

> a. Phase out the non-therapeutic use in livestock of medically important antibiotics;
>
> b. Require this same tough standard of new applications for approval of antibiotics;
>
> c. Provisions for the therapeutic use of antibiotics in the treatment of sick animals, treat pets and other animals not used for food consumption.(GovTrak, 2009)

The PAMTA would prove to be a monumental step in the fight against antibiotic resistance in clinical infections. Ultimately, the analysis from this longitudinal study examining the organic-transition process may provide vital scientific knowledge to undergird such legislative action and spur necessary change in U.S. public health policy regarding antibiotic use within the industrial food animal production complex.

IV. CONCLUSIONS

In summation, the results of this baseline study confirm the prevalence and current levels of susceptible and antibiotic resistant *Enterococcus* spp. and *Salmonella* spp. in conventional and organic-transitioning poultry farm environments. These findings suggest that production management practices may play a role in the prevalence and antibiotic resistance of selected bacterial species within differing poultry production operations. In addition, the influence of environmental factors within the environmental-microbial-resistance paradigm was explored with respect to production practice. Our study findings demonstrate the initial effects of an ecosystem-level intervention to reduce the prevalence of antimicrobial resistance in food-borne bacteria derived from farm environments via modification in production practice. In addition, this baseline study establishes the foundation for future comparative work examining antibiotic resistance in differing poultry production environments over time. To conclude, this on-farm intervention study will contribute to the growing body of knowledge in examining the food-borne bacteria and antibiotic resistance patterns in food animal production environments as a measure of organic production practice adoption.

List of Tables

Table 1: Clinical and Laboratory Standards Institute Interpretative Criteria for MIC determinations of *Enterococcus* spp. and *Salmonella* spp.

Antimicrobial Class	Antimicrobial Agent	Tested range (ug/ml)	Resistance Breakpoint[a]
Aminoglycosides			
	$Amikacin_S$	0.25-64	≥32
	$Gentamicin_E$	64-2048	≥500
	$Gentamicin_S$	0.125-16	≥16
	$Kanamycin_E$	64-2048	≥512
	$Kanamycin_S$	64-2048	≥64
	$Streptomycin_E$	256-2048	>1000
	$Streptomycin_S$	16-64	≥64
Bambermycin			
	$Flavomycin_S$	0.5-32	>32
B-lactam/ Blactamase inhibitors combinations			
	Augmentin (amoxicillin-clavulanic acid)$_S$	0.5-32	≥16
	$Ampicillin_S$	0.5-32	≥32
Cephems			
	$Cefoxtin_S$	0.25-32	≥32
	$Ceftiofur_S$	0.06-16	≥8
	$Ceftriaxone_S$	0.25-64	≥64

Table 1: (cont'd)

Chloramphenicol			
	$Chloramphenicol_E$	1-32	≥32
	$Chloramphenicol_S$	1-32	≥32
Folate pathway inhibitors			
	$Trimeth/sulfameth_S$	0.125-8	≥4/76
Glycylcyclines			
	$Tigecycline_E$	0.015-0.05	≥0.25[b]
Glycopeptides; glycopeptide			
	$Vancomycin_E$	0.25-32	≥32
Lincosamides			
	$Lincomycin_E$	0.05-32	≥8
Lipopeptides			
	$Daptomycin_E$	0.25-16	≥4b
Macrolides			
	$Erythromycin_E$	0.25-8	≥8
	$Tylosin_E$	0.125-32	≥32
Nitrofurans			
	$Nitrofurantoin_E$	1-64	≥64
Oxazolidinones			
	$Linezolid_E$	0.25-8	≥8
Pencillins			
	$Penicillin_E$	0.25-16	≥16
Streptogramins (combo)			
	$Quinupristin/dalfopristin_E$	0.5-32	≥4
Sulfathiazole			
	$Sulfisoxazole_S$	16-512	≥512
Tetracyclines			
	$Tetracycline_E$	0.25-32	≥16
	$Tetracycline_S$	4-32	≥16

Table 1: (cont'd)

Quinolones			
	Ciprofloxacin$_E$	0.06-4	≥4
	Ciprofloxacin$_S$		
	Nalidixic Acid$_S$	0.25-32	≥32

[a] All resistance breakpoints are those defined by CLSI unless otherwise noted.

[b] For daptomycin and tigecycline, resistance breakpoint has not been established. Report as non-susceptible.

[E] Antimicrobial agents tested on *Enterococcus* spp.

[S] Antimicrobial agents tested on *Salmonella* spp.

Table 2: Most common *Salmonella* spp. serovars isolated from Humans

RANKING	*SALMONELLA* SEROTYPE
1	Typhimurium
2	Enteritidis
3	Newport
4	Heidelberg
5	Javiana
6	Montevideo
7	Braenderup
8	Muechen
9	Saintpaul
10	Paratyphi B[1]

Source: Adapted from Most Common Serotypes among ***Salmonella*(non-Typhi) Isolates from Humans, Retail Meats, and Food Animals, 2005**
available at *www.fda.gov/cvm/Documents/**2005**NarmsExeRptT5.pdf*

Table 3: Organic Poultry Production in top four states from 2000-2005

Top four States for organic poultry production, 2000 and 2005

State	Animals produced in 2000	Share of U.S. organic production	State	Animals produced in 2005	Share of U.S. organic production
	Number	*Percent*		*Number*	*Percent*
Broilers			*Broilers*		
California	1,200,000	62	California	3,567,425	34
North Carolina	410,242	21	Pennsylvania	2,880,800	28
Oklahoma	140,000	7	Nebraska	2,435,546	23
Iowa	69,170	4	Iowa	886,280	9
Total	1,819,412	94	Total	9,770,051	94
Layer hens			*Layer hens*		
North Carolina	462,576	42	North Carolina	301,500	15
Pennsylvania	148,079	13	California	281,070	14
California	116,608	10	Pennsylvania	273,986	13
Virginia	93,680	8	Iowa	242,526	12
Total	820,943	73	Total	1,099,082	54
Turkeys			*Turkeys*		
California	7,664	84	Michigan	56,729	39
New Mexico	500	5	Pennsylvania	48,815	34
Ohio	210	2	California	18,025	13
Iowa/Pennsylvania (T)	200	2	Iowa	15,260	11
Total	8,574	93	Total	138,829	97

Source: USDA, ERS, 2006b: Organic Agricultural Production in 2005.
www.ers.usda.gov/data/organic/

Table 4: Selected antimicrobials approved by the FDA for use in broiler production

Antibiotics Used in Poultry		Labeled as a Growth Promoter	Example of Human Drug Exhibiting Complete Cross-resistance
Aminoglycosides			
	Streptomycin	No	Kanamycin, Neomycin
	Neomycin	No	Kanamycin
	Gentamicin	No	None
Aminocyclitols			
	Spectinomycin	Yes	None
B-lactams			
	Penicillin	Yes	Ampicillin
Decapeptides			
	Bacitracin	Yes	Bacitracin
Fluoroquinolones			
	Enrofloxacin	No	Ciprofloxacin
	Sarafloxacin	No	None
Lincosamides			
	Lincomycin	No	Clindamycin
Macrolides			
	Erythromycin	No	Clarithromycin,Azithromycin
	Tylosin	Yes	Erythromycin
Tetracyclines			
	Chlortetracycline	Yes	Oxytetracycline,Tetracycline,
	Oxytetracycline	No	Chlortetracycline,Tetracycline
	Tetracycline	No	Oxytetracycline, Chlortetracycline
Streptogramins			
	Virginiamycin	Yes	Quinupristin/Dalfopristin
Bambermycin		Yes	None
Novobiocin		No	None
Oleandomycin		Yes	Erythromycin

Table 5: Poultry House Demographics for study Conventional and Organic-transitioning poultry house

Poultry House Characteristics	Mean		50th percentile		90th percentile	
	Organic-transitioning (n=10)	Conventional (n=10)	Organic-transitioning	Conventional	Organic-transitioning	Conventional
Organic Months	1.71	0	1	0	3.55	0
Temperature (outside)	59.53	68.99	55	67	74	80
Temperature (inside)	68.27	73.38	68	73	75	77.5
Humidity (outside)	57.02	53.9	47.5	48	99.9	73.5
Humidity (inside)	68.6	69.5	68.5	69	90	89.5
Airflow	0.33	0.703	0.4	0.5	0.7	1.9
Water Activity (A_w)	0.829	0.838	0.85	0.855	0.965	0.95
Ambient Light, Fans on	7.26	7.1	3.575	5.53	16.7	13.41
Ambient Light, Fans off	2.23	.	1.93	.	3.1	.
Length of house	500	411	500	500	500	500
Width of house	46.8	44.6	48	44	48	50
Depth of poultry Litter	5.4	4.8	5	4	7	8
Months after litter change	4	2.14	2.33	4	12	8
Number of chickens	22550	30800	24000	30800	24000	30800
Age of flock	35.8	36.1	35	36	40.5	40
Mortality rate	4.704	2.56	4.475	2.5	6.9	2.6
Minutes outdoors	0	0	0	0	0	0

Table 6: *Enterococcus* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms

	Poultry House Type		
Environmental Source	**TOTAL (n=260)**	**ORGANIC-TRANSITIONING (n=126)**	**CONVENTIONAL (n=134)**
Poultry Litter (1)	64(25.0)	35(28)	29(22.1)
Poultry Litter (2)	61(23.4)	30(24)	31(23)
Poultry Litter (3)	60(23.1)	30(24)	30(22.3)
Poultry Feed	57(22)	27(21.4)	30(22.3)
Water (Source)	2(.8)	1(.8)	1(.75)
Water (Waterline)	16(6.2)	3(2.4)	13(9.7)

Table 7: Distribution of *Enterococcus* species isolated from organic-transitioning and conventional poultry production systems

	Poultry House Type		
Species Identification	**TOTAL (n=260)**	**ORGANIC-TRANSITIONING (n=126)**	**CONVENTIONAL (n=134)**
Enterococcus durans	9 (3.46)	7(5.55)	2(1.49)
Enterococcus durans/hirae	1(.38)	1(.79)	0
Enterococcus faecalis	122(47)	63(50)	55(41)
Enterococcus faecium	131(50.4)	42(33.3)	70(52.2)
Enterococcus gallinarium	7(2.7)	5(3.97)	2(1.49)
Enterococcus gallinarium/faecium	1(.38)	1(.79)	0
Enterococcus hirae	12(4.62)	7(5.55)	5(3.73)

Table 8: Distribution of *Enterococcus* species isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms

Type of House	Species				
	E.durans	*E.faecalis*	*E.faecium*	*E.gallanarium*	*E.hirae*
Conventional					
Poultry Litter 1 (n=29)	0	8(28.6)	20(68.5)	1(3.45)	0
Poultry Litter 2 (n=31)	0	16(51.6)	15(48.38)	0	0
Poultry Litter 3 (n=30)	0	10(33.3)	16(53.3)	1(3.33)	3(10)
Poultry Feed (n=29)	0	10(34.5)	15(51.7)	1(3.45)	3(10.3)
Water: Source (n=1)	1(100)	0	0	0	0
Water: Waterline (n=13)	0	0	12(92.3)	0	1(7.69)
Organic-Transitioning					
Poultry Litter 1 (n=35)	0	20(57.1)	9(25.7)	4(11.4)	2(5.71)
Poultry Litter 2 (n=30)	6(20)	16(53.3)	8(26.7)	0	0
Poultry Litter 3 (n=30)	0	27(90)	1(3.33)	0	1(3.33)
Poultry Feed (n=27)	1(3.57)	0	22(81.4)	0	4(14.3)
Water: Source (n=1)	0	0	1(100)	0	0
Water: Waterline (n=3)	1(33.3)	0	1(33.3)	1(33.3)	0

Table 9: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus* spp. (n=260) collected from conventional and organic-transitioning poultry farms

Enterococcus spp. (n=260)

Antimicrobial Agent	Production Practice	No. of Isolates MIC (ug/ml) of: 0.015	0	0.1	0.1	0.3	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048	MIC Range (ug/ml)
Chloramphenicol																					
	Conventional								0	61	69	2	2[a]								2->32
	Organic-Transitioning								1[b]	31	89	5	0								2-16
Ciprofloaxcin																					
	Conventional					2	8	61	20	43[a]											0.25->4
	Organic-Transitioning					3	14	53	41	13[a]											0.25->4
Daptomycin																					
	Conventional						11[b]	53	29	41											≤0.5-4
	Organic-Transitioning						11[b]	55	42	19											≤0.5-4
Erythromycin																					
	Conventional						40[b]	19	16	8	51[a]										≤0.5->8
	Organic-Transitioning						39[b]	28	23	14	20[a]										≤0.5->8
Flavomycin																					
	Conventional							2[b]	50	5	12	63[a]									≤1->16
	Organic-Transitioning							31[b]	36	2	1	56[a]									≤1->16
Gentamicin																					
	Conventional													112[b]	5	5	12[a]				≤128->1024
	Organic-Transitioning													126[b]	0	0	0				128
Kanamycin																					
	Conventional													83[b]	19	0	32[a]				≤128->1024
	Organic-Transitioning													93[b]	24	1	8[a]				≤128->1024
Lincomycin																					
	Conventional							3[b]	0	3	5	16	107[a]								≤1->32
	Organic-Transitioning							7[b]	2	4	7	35	71[a]								≤1->32
Linezolid																					
	Conventional						5[b]	63	63	2	1										≤0.5-8
	Organic-Transitioning						1[b]	27	76	22	0										≤0.5-4

Table 9: (cont'd)

Enterococcus spp. (n=260)

Antimicrobial Agent	Production Practice	No. of Isolates MIC (ug/ml) of: 0.015	0	0.1	0.1	0.3	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048	MIC Range (ug/ml)
Nitrofurantoin																					
	Conventional										29	16	9	80[a]							8-≥64
	Organic-Transitioning										24	24	10	68[a]							8-≥64
Penicillin																					
	Conventional						6[b]	7	13	53	13	42[a]									≤0.5-≥16
	Organic-Transitioning						6[b]	7	14	79	19	1									≤0.5-≥16
Streptomycin																					
	Conventional																111[b]	9	7	7	≤512-≥2048
	Organic-Transitioning																116[b]	4	3	3	≤512-≥2048
Quinupristin/ Dalfopristin																					
	Conventional							11[b]	18	73	21	6	5								≤1-32
	Organic-Transitioning							13[b]	42	29	40	2	0								≤1-16
Tetracycline																					
	Conventional									15[b]	2	6	111[a]								≤4-≥32
	Organic-Transitioning									45[b]	4	0	76[a]								≤4-≥32
Tigecycline																					
	Conventional		2	31	58	36	7														0.03-0.5
	Organic-Transitioning		4	26	49	47	0														0.03-0.25
Tylosin																					
	Conventional							10	23	37	9	4	51[a]								1-≥32
	Organic-Transitioning							3	38	46	20	1	18[a]								1-≥32
Vancomycin																					
	Conventional						53[b]	54	24	3											≤0.5-4
	Organic-Transitioning						31[b]	66	24	5											≤0.5-4

[1] For daptomycin and tigecycline represents number non-susceptible

[a] Number of isolates with MICs gretater than or equal to the highest concentration on Sensititre plate

[b] Number of isolates with MICs less than or equal to the lowest tested concentration on Sensititre plate

Table 10: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus faecalis (*n=180) collected from conventional and organic-transitioning poultry farms

Enterococcus faecalis (n=118)

Antimicrobial Agent	Production Practice	No. of Isolates by MIC (ug/ml) of:																		MIC Range (ug/ml)
		0.015	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Chloramphenicol																				
	Conventional								0	22	30	1	2[a]							4-≥32
	Organic-Transitioning								0	6	52	5	0							4-16
Ciprofloaxcin																				
	Conventional					0	3	44	5	3										0.5-4
	Organic-Transitioning					0	0	34	26	3[a]										1-≥4
Daptomycin																				
	Conventional						6[b]	45	0	4										≤0.5-4
	Organic-Transitioning						2[b]	51	8	2										≤0.5-4
Erythromycin																				
	Conventional						5[b]	5	8	0	37[a]									≤0.5->8
	Organic-Transitioning						17[b]	25	8	0	11[a]									≤0.5->8
Flavomycin																				
	Conventional							2[b]	46	1	1	5[a]								≤1->16
	Organic-Transitioning							31[b]	31	0	0	1[a]								≤1->16
Gentamicin																				
	Conventional														50[b]	0	1	4[a]		≤128->1024
	Organic-Transitioning														63[b]	0	0	0		128
Kanamycin																				
	Conventional														42[b]	1	0	12[a]		≤64->1024
	Organic-Transitioning														57[b]	1	0	5[a]		≤64->1024
Lincomycin																				
	Conventional							0	0	0	0	7	48[a]							1->32
	Organic-Transitioning							0	0	0	0	13	50[a]							1->32
Linezolid																				
	Conventional						2[b]	42	11	0	0									≤0.5-2
	Organic-Transitioning						0	11	39	13	0									1-4

Table 10: (cont'd)

Enterococcus faecalis (n=118)

Antimicrobial Agent	Production Practice	No. of Isolates MIC (ug/ml) of: 0.015	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048	MIC Range (ug/ml)
Nitrofurantoin																					
	Conventional										29	16	1	9[a]							8->64
	Organic-Transitioning										24	20	4	15[a]							8->64
Penicillin																					
	Conventional						0	0	8	40	4	3[a]									2->16
	Organic-Transitioning						0	0	2	53	8	0									2-8
Streptomycin																					
	Conventional																38[b]	6	6	5	≤512->2048
	Organic-Transitioning																56[b]	2	2	3	≤512->2048
Quinupristin Dalfopristin																					
	Conventional							0	1	37	10	5	2								2-64
	Organic-Transitioning							0	1	24	38	0	0								2-8
Tetracycline																					
	Conventional									2[b]	0	4	49[a]								≤4->32
	Organic-Transitioning									0	0	0	63[a]								≥32
Tigecycline																					
	Conventional		2	11	19	18	5														0.03-0.5
	Organic-Transitioning		0	11	18	34	0														0.06-0.25
Tylosin																					
	Conventional							3	8	4	1	0	39[a]								1->32
	Organic-Transitioning							3	22	22	3	0	13[a]								1->32
Vancomycin																					
	Conventional						2[b]	31	19	3											≤0.5-4
	Organic-Transitioning						0	39	20	4											1-4

[1] For daptomycin and tigecycline represents number non-susceptible

[a] Number of isolates with MICs gretater than or equal to the highest concentration on Sensititre plate

[b] Number of isolates with MICs less than or equal to the lowest tested concentration on Sensititre plate

Table 11: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 17 antimicrobials among *Enterococcus faecium (*n=113) collected from conventional and organic-transitioning poultry farms

Enterococcus faecium (n=113)

Antimicrobial Agent	Production Practice	No. of Isolates associated with MIC (ug/ml) of:																			MIC Range (ug/ml)
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048	
Chloramphenicol																					
	Conventional								0	33	36	1	0								4-16
	Organic-Transitioning								1	14	27	0	0								2-8
Ciprofloaxcin																					
	Conventional					1	1	16	12	40[a]											0.25-≥4
	Organic-Transitioning					0	3	15	15	9[a]											0.5-≥4
Daptomycin																					
	Conventional						3[b]	6	25	36											≤0.5-4
	Organic-Transitioning						3[b]	2	26	11											≤0.5-4
Erythromycin																					
	Conventional						33[b]	13	8	7	9[a]										≤0.5-≥8
	Organic-Transitioning						12[b]	3	13	10	4[a]										≤0.5-≥8
Flavomycin																					
	Conventional							0	2	4	11	52[a]									2-≥16
	Organic-Transitioning							0	3	2	0	37[a]									2-≥16
Gentamicin																					
	Conventional														53[b]	5	4	8[a]			≤128-≥1024
	Organic-Transitioning														42[b]	0	0	0			128
Kanamycin																					
	Conventional														34[b]	18	0	18[a]			≤128-≥1024
	Organic-Transitioning														20[b]	19	1	2			≤128-1024
Lincomycin																					
	Conventional							3[b]	0	3	5	6	53[a]								≤1->32
	Organic-Transitioning							6[b]	2	2	3	16	14[a]								≤1->32
Linezolid																					
	Conventional						0	16	51	2	1										≤1-8
	Organic-Transitioning						0	7	27	8	0										≤1-4

Table 11: (cont'd)

Enterococcus faecium (n=113)

Antimicrobial Agent	Production Practice	No. of Isolates associated with MIC (ug/ml) of:																			MIC Range (ug/ml)
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048	
Nitrofurantoin																					
	Conventional										0	0	5	65[a]							32->64
	Organic-Transitioning										0	3	2	37[a]							16->64
Penicillin																					
	Conventional						4[b]	6	2	12	9	37[a]									≤0.5->16
	Organic-Transitioning						2[b]	1	9	22	7	1									≤0.5->16
Streptomycin																					
	Conventional																64[b]	3	1	2	≤512->2048
	Organic-Transitioning																41[b]	0	1	0	≤512-2048
Quinupristin Dalfopristin																					
	Conventional							11[b]	12	32	11	1	3								≤1-32
	Organic-Transitioning							10[b]	24	5	2	1	0								≤1-16
Tetracycline																					
	Conventional									11[b]	2	2	55[a]								≤4->32
	Organic-Transitioning									32[b]	4	0	5[a]								≤4->32
Tigecycline																					
	Conventional		0	19	34	16	1														0.06-0.5
	Organic-Transitioning		1	13	20	8	0														0.03-0.125
Tylosin																					
	Conventional							6	14	31	8	4	7[a]								1->32
	Organic-Transitioning							0	8	18	15	1	0								2-16
Vancomycin																					
	Conventional						48[b]	17	5	0											≤0.5-2
	Organic-Transitioning						18[b]	19	4	1											≤0.5-4

[1] For daptomycin and tigecycline represents number non-susceptible

[a] Number of isolates with MICs gretater than or equal to the highest concentration on Sensititre plate

[b] Number of isolates with MICs less than or equal to the lowest tested concentration on Sensititre plate

Table 12: *Salmonella* spp. isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms

	Poultry House Type		
Environmental Source	**TOTAL (n=119)**	**ORGANIC-TRANSITIONING (n=76)**	**CONVENTIONAL (n=24)**
Poultry Litter (1)	29 (24.4)	26(34.2)	3(12.5)
Poultry Litter (2)	38(31.9)	28(36.8)	3(12.5)
Poultry Litter (3)	39(32.8)	22(28.9)	6(25)
Poultry Feed	9(7.56)	0	9(37.5)
Water (Source)	0	0	0
Water (Waterline)	0	0	0
Soil	3(2.52)	0	3(12.5)

Table 13: Distribution of *Salmonella* species isolated from organic-transitioning and conventional poultry production systems

Species Identification	Poultry House Type		
	TOTAL (n=119)	ORGANIC-TRANSITIONING (n=76)	CONVENTIONAL (n=24)
Salmonella enteritidis	17 (14.3)	14(18.4)	0
Salmonella gostrup	6(5.04)	6(7.9)	0
Salmonella infantis	6(5.04)	6(7.9)	0
Salmonella kentucky	76(63.9)	49(64.5)	12(50)
Salmonella orion	12(10.1)	0	12(50)
Salmonella typhmirum	1(.08)	0	0
Salmonella spp. *(unidentified)*	1(.08)	1(1.32)	0

Table 14: Distribution of *Salmonella* species isolated from water, poultry litter, and poultry feed samples collected from conventional and organic-transitioning poultry farms

House Type	Species				
	S.enteritidis	*S.gostrup*	*S.infantis*	*S.kentucky*	*S.orion*
Conventional					
Poultry Litter 1 (n=12)	0	0	0	12(100)	0
Poultry Litter 2 (n=9)	0	0	0	0	9(100)
Poultry Litter 3 (n=0)	0	0	0	0	0
Poultry Feed (n=0)	0	0	0	0	0
Water: Source (n=3)	0	0	0	0	3(100)
Water: Waterline (n=0)	0	0	0	0	0
Organic-Transitioning					
Poultry Litter 1 (n=76)	14(18.4)	6(7.9)	6(7.9)	49(64)	0
Poultry Litter 2 (n=0)	0	0	0	0	0
Poultry Litter 3 (n=0)	0	0	0	0	0
Poultry Feed (n=0)	0	0	0	0	0
Water: Source (n=0)	0	0	0	0	0
Water: Waterline (n=0)	0	0	0	0	0

Table 15: Minimal inhibitory concentration (MIC) distributions (μg/ml) for 15 antimicrobials among *Salmonella* spp. (n=120) collected from conventional and organic-transitioning poultry farms

Salmonella spp. (n=100)

Antimicrobial Agent	Production Practice	No. of Isolates by MIC (ug/ml) of:																	MIC Range (ug/ml)
		0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amikacin																			
	Conventional							0	17	4	3								2-8
	Organic-Transitioning							31	36	6	3								1-8
Augmentin																			
	Conventional							19[b]	0	0	0	0	5						1-32
	Organic-Transitioning							69[b]	0	2	0	2	3[a]						1-32
Ampicillin																			
	Conventional							18[b]	1	0	0	0	5						1-32
	Organic-Transitioning							57[b]	16	0	0	0	3[a]						1-32
Cefoxitin																			
	Conventional								10	9	5[a]								2-8
	Organic-Transitioning								47	21	5		3						2-32
Ceftiofur																			
	Conventional						3	16	0	0	5								0.5-8
	Organic-Transitioning						20	46	6	1	3[a]								0.5-8
Ceftriaxone																			
	Conventional					19[b]	0	0	0	0	0	4	1						0.25-32
	Organic-Transitioning					72[b]	1	0	0	0	0	3	0						0.25-16
Chloramphenicol																			
	Conventional									16	8								4-8
	Organic-Transitioning									34	42								4-8

Table 15: (cont'd)

Salmonella spp. (n=100)

Antimicrobial Agent	Production Practice	No. of Isolates by MIC (ug/ml) of: 0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	MIC Range (ug/ml)
Ciprofloxacin																			
	Conventional	21[b]	2	0	0	1													0.015-0.25
	Organic-Transitioning	59[b]	16		1														0.015-0.125
Gentamicin																			
	Conventional						12	9	3										≤0.5-2
	Organic-Transitioning					22[b]	44	6	2	2									≤0.25-4
Kanamycin																			
	Conventional										24[b]								8
	Organic-Transitioning										74[b]			1					8-64
Naladixic acid																			
	Conventional								12	11									2-4
	Organic-Transitioning								36	38	2								2-8
Streptomycin																			
	Conventional												14[b]	10					32-64
	Organic-Transitioning												26[b]	50[a]					32-64
Sulfisoxazole																			
	Conventional												5	19					32-64
	Organic-Transitioning												32	44					32-64
Tetracycline																			
	Conventional												14[b]	10					32-64
	Organic-Transitioning									25[b]	1		50[a]						2-32
Trimethoprim Sulphamethoxazole																			
	Conventional				24[b]														0.125
	Organic-Transitioning				76[b]														0.125

[a] Number of isolates with MICs gretater than or equal to the highest concentration on Sensititre plate

[b] Number of isolates with MICs less than or equal to the lowest tested concentration on Sensititre plate

Table 16: Multi-drug antibiotic resistance profiles of *Salmonella* spp. isolated from conventional and organic-transitioning poultry farm samples

Antimicrobial resistance profile	Serovar Kentucky	
	Organic (n=76)	Conventional (n=24)
AUG-AMP-FOX-TIO-STR-TET	3 (3.94)	5 (21)
STR-TET	47(62)	5(21)
Susceptible to all tested antimicrobials	26(34.2)	14(58)

Table 17: Correlation Table of Environmental Variables associated with Conventional and Organic-Transitioning Poultry Houses adjusted by Intra-Poultry House and Intra-Farm Variation

	House Type	Temperature Outside	Humidity Outside	Temperature Inside	Humidity Inside	Antibiotic Feed	Rain	Antibiotic Water	Antibiotic Hatchery	Vaccine Hatchery	Antibiotic Breeder	Vaccine Breeder	Cloud Cover	Distance from Nearest Conv. Farm
Type of House	1.00													
Temperature Outside	0.45	1.00												
Humidity Outside	-0.04	-0.47	1.00											
Temperature Inside	0.58	0.70	-0.29	1.00										
Humidity Inside	0.04	-0.42	0.77	-0.37	1.00									
Antibiotics (Feed)	1.00	0.45	-0.04	0.58	0.04	1.00								
Rain	-0.41	-0.48	0.52	-0.43	0.41	-0.41	1.00							
Antibiotics (Water)	0.32	-0.07	-0.21	0.15	0.22	0.32	-0.13	1.00						
Antibiotics (Hatchery)	1.00	0.45	-0.04	0.58	0.04	1.00	-0.41	0.32	1.00					
Vaccine (Hatchery)	0.36	-0.30	0.38	0.20	0.28	0.36	0.14	0.11	0.36	1.00				
Antibiotics (Breeder)	0.33	0.60	-0.17	0.44	-0.20	0.33	-0.34	0.10	0.33	-0.11	1.00			
Vaccine (Breeder)	0.50	0.68	-0.09	0.67	-0.17	0.50	-0.15	0.16	0.50	-0.17	0.65	1.00		
Cloud Cover	-0.12	-0.61	0.68	-0.50	0.77	-0.12	0.45	0.11	-0.12	0.45	-0.36	-0.56	1.00	
Distance from Nearest Conventional Farm	0.83	0.51	-0.36	0.54	-0.22	0.83	-0.72	0.26	0.83	0.31	0.47	0.28	-0.21	1.00

List of Figures

Figure 1: Photograph of Sensititre™ antimicrobial susceptibility testing system (Trek Diagnostic Systems, Westlake, Ohio)

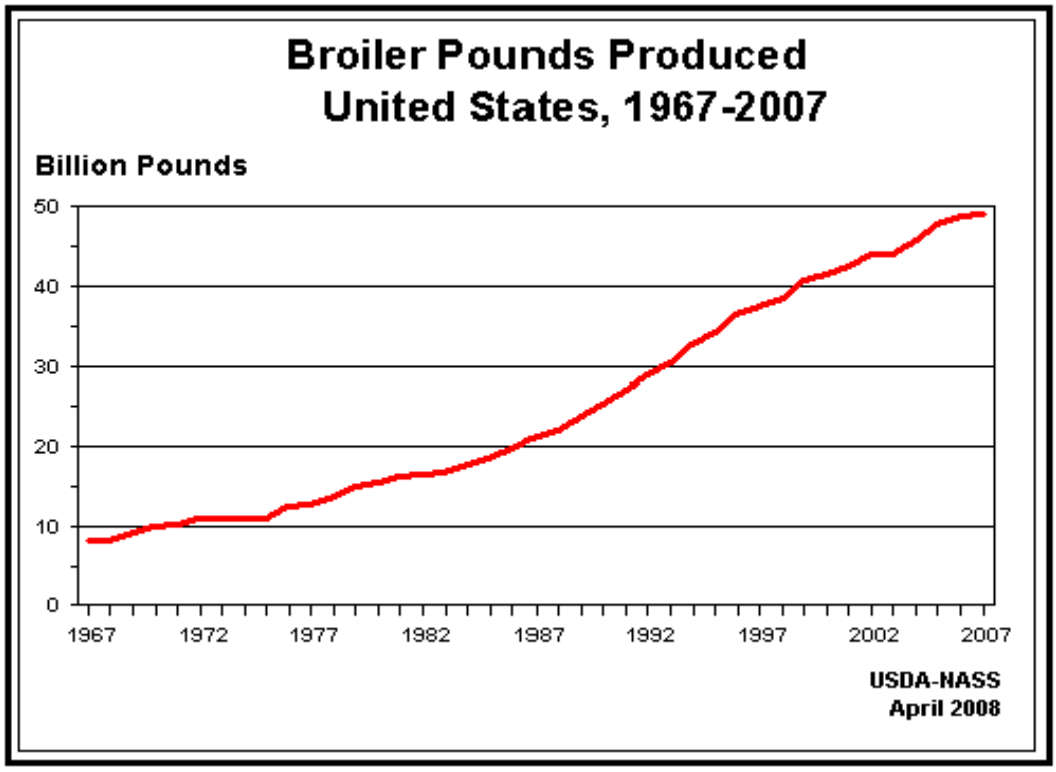


Figure 2: U.S. boiler production from 1967-2007 (billion pounds)

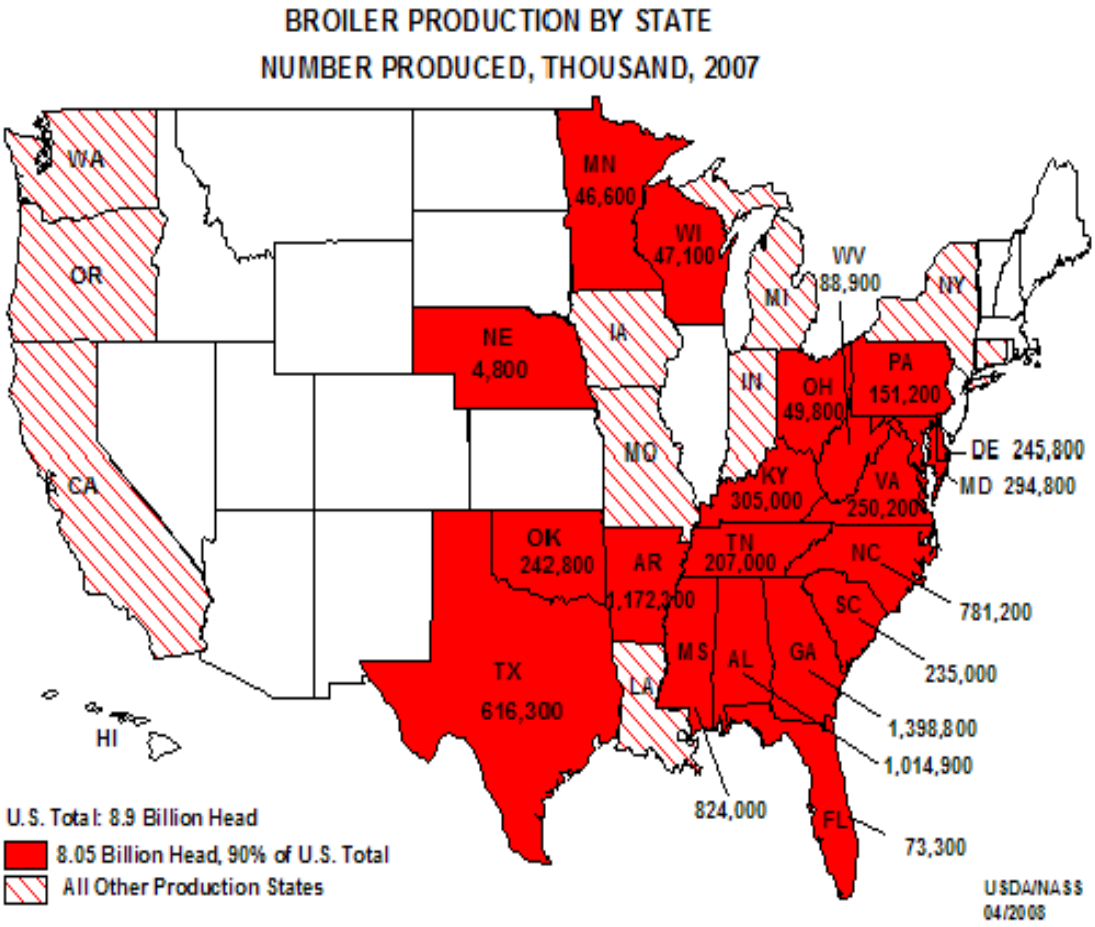


Figure 3: U.S. Broiler Production by State in 2007, (number produced thousand)

Poultry House Types: Transitioning to Organic (n=10), Conventional Control (n=10)

Conventional Controls

Transitional to Organic

Figure 4: Photographic depiction of typical conventional and organic poultry houses.

Organic Food Categories	Sales ($Mil)	% Growth 2005
Dairy	2,140	23.6%
Bread & Grains	1,360	19.2%
Beverages (incl. non-dairy)	1,940	13.2%
Fruit & Vegetables	5,369	10.9%
Snack Foods	667	18.3%
Packaged/Prepared Foods	1,758	19.4%
Sauces/Condiments	341	24.2%
Meat/Fish/Poultry	256	55.4%
Total Org Consumer Food Sales	**13,831**	**16.2%**

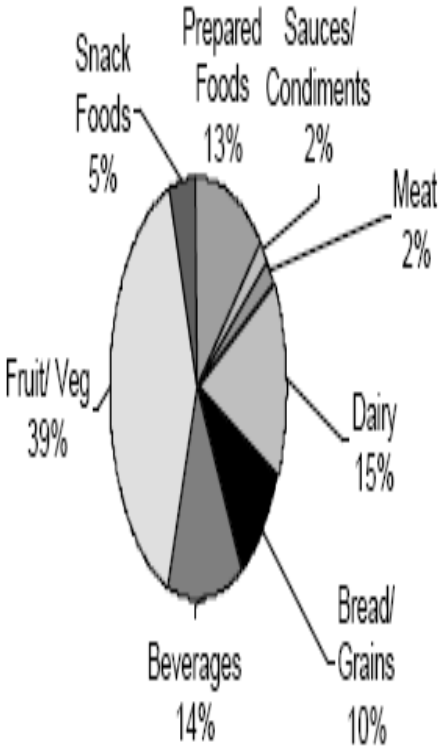
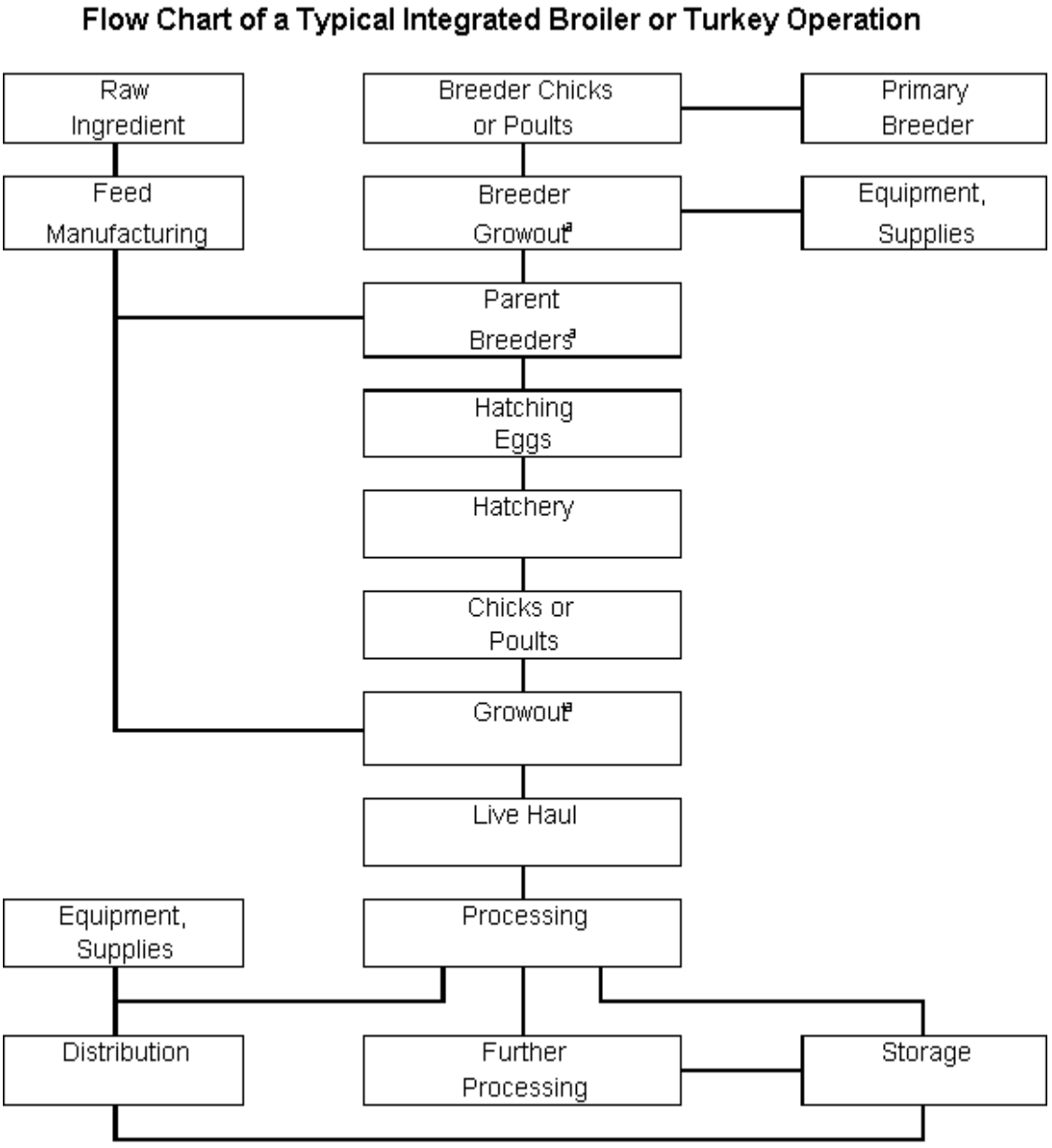
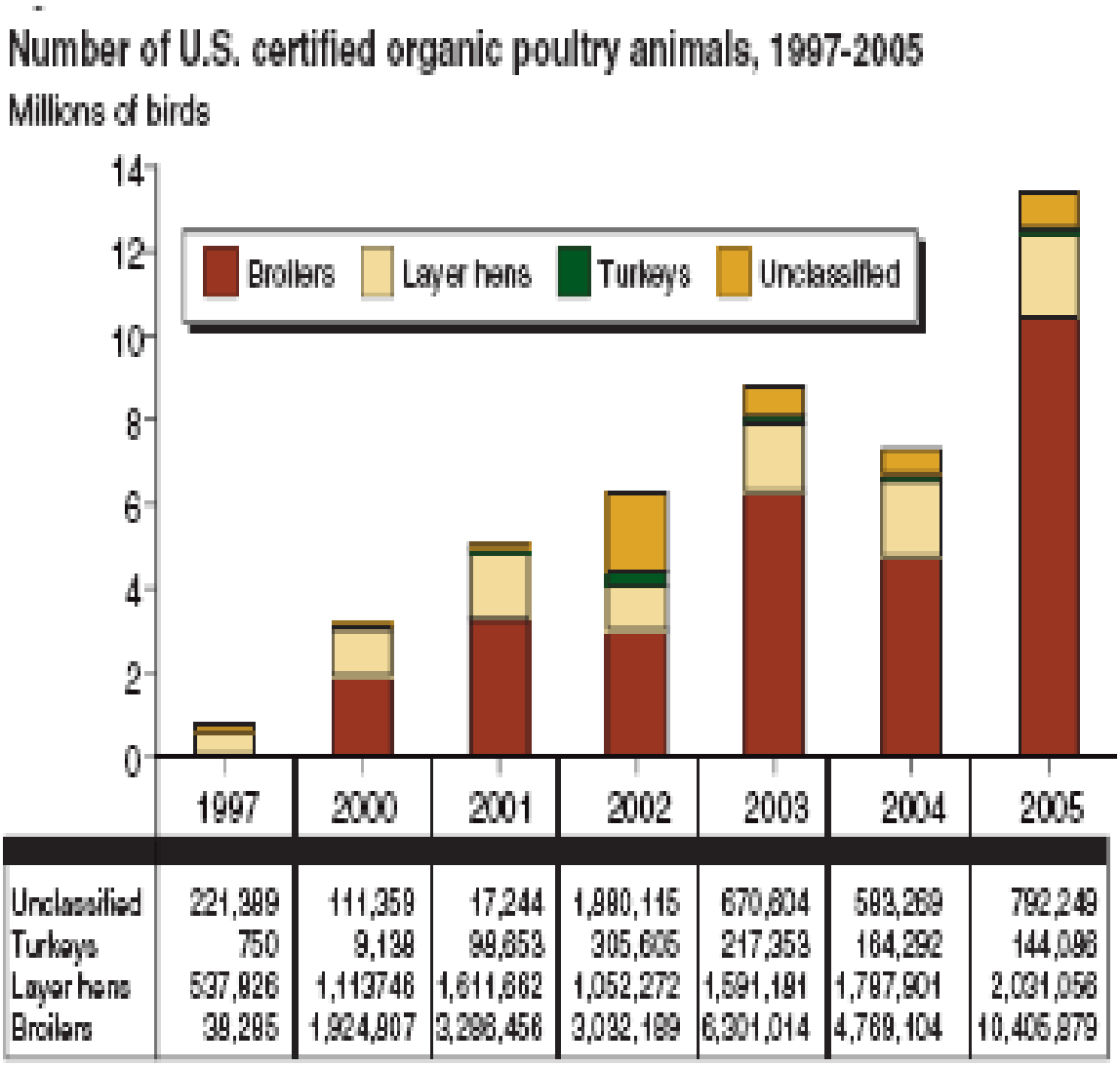


Figure 5: Organic food share market in 2005; Source: OTA's 2006 Manufacturer Survey:available at www.ota



a. Normally produced under contract arrangement with grower

Figure 6: Schematic of Vertical Integration within the Broiler Production Industry



	1997	2000	2001	2002	2003	2004	2005
Unclassified	221,389	111,359	17,244	1,980,115	670,804	583,269	792,249
Turkeys	750	9,138	99,853	305,605	217,353	164,292	144,096
Layer hens	537,826	1,113746	1,611,882	1,052,272	1,591,181	1,797,901	2,031,056
Broilers	38,285	1,924,907	3,286,456	3,032,199	6,301,014	4,769,104	10,406,879

Figure 7: Number of U.S. certified organic poultry animals, 1997-2005; Source: USDA, Economic Research Service, 2006b: Organic Agricultural Production in 2005. Available at www.ers.usda.gov/data/organic/.

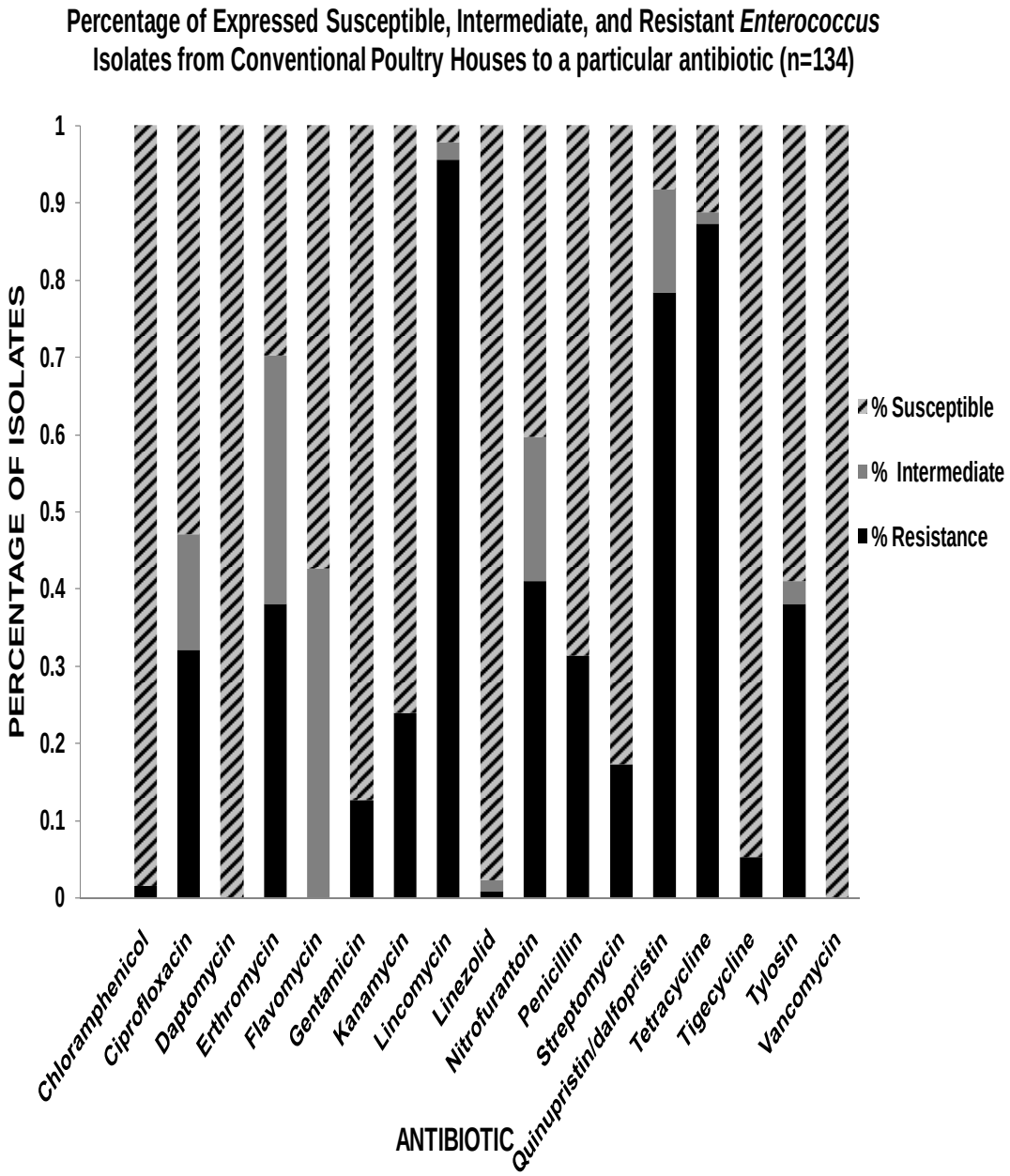


Figure 8: Percentage of Expressed Susceptible, Intermediate, and Resistant *Enterococcus* spp. Isolates from Conventional Poultry Houses to a particular antibiotic (n=134)

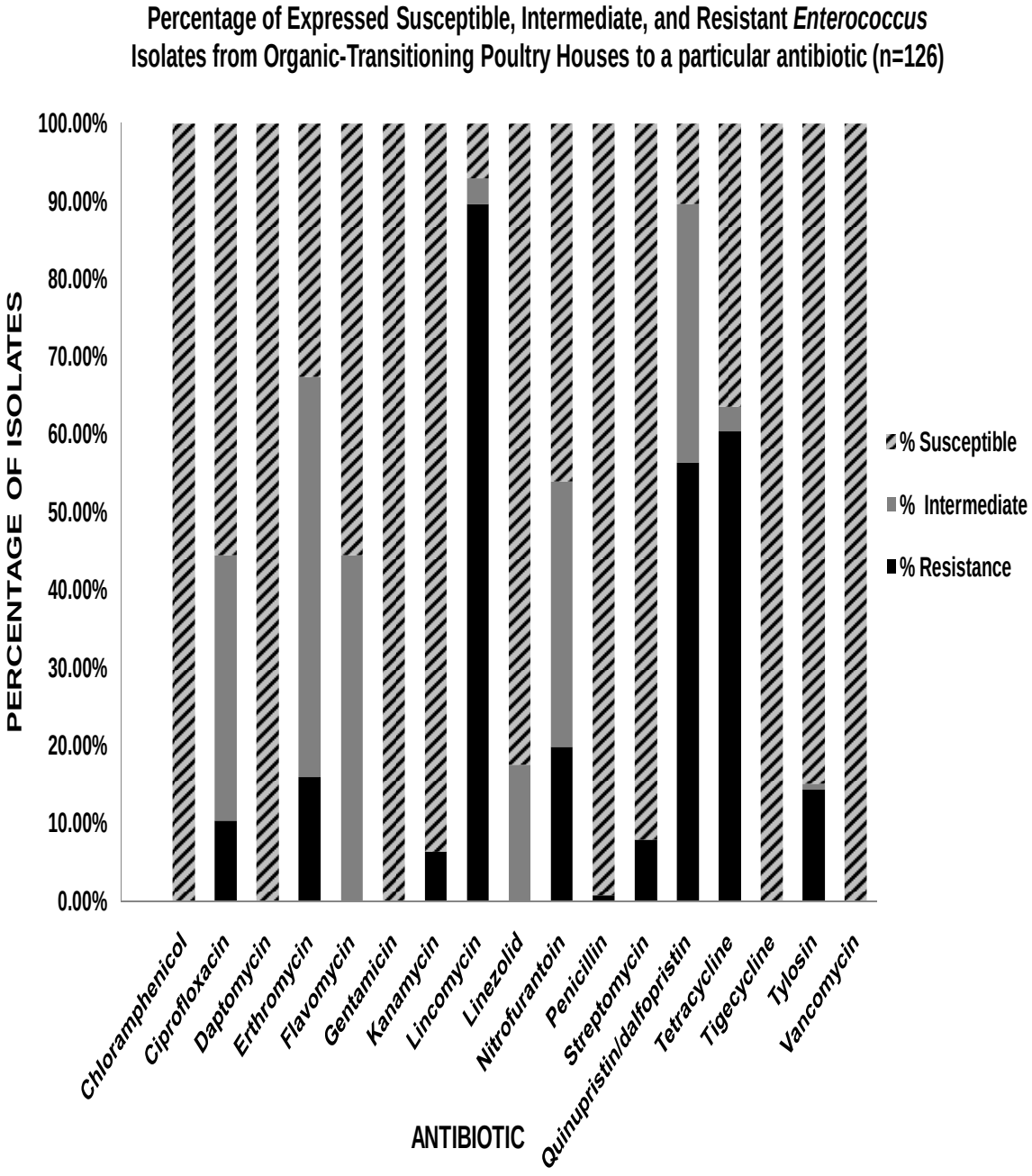


Figure 9: Percentage of Expressed Susceptible, Intermediate, and Resistant *Enterococcus* spp. Isolates from Organic-Transitioning Poultry Houses to a particular antibiotic (n=126)

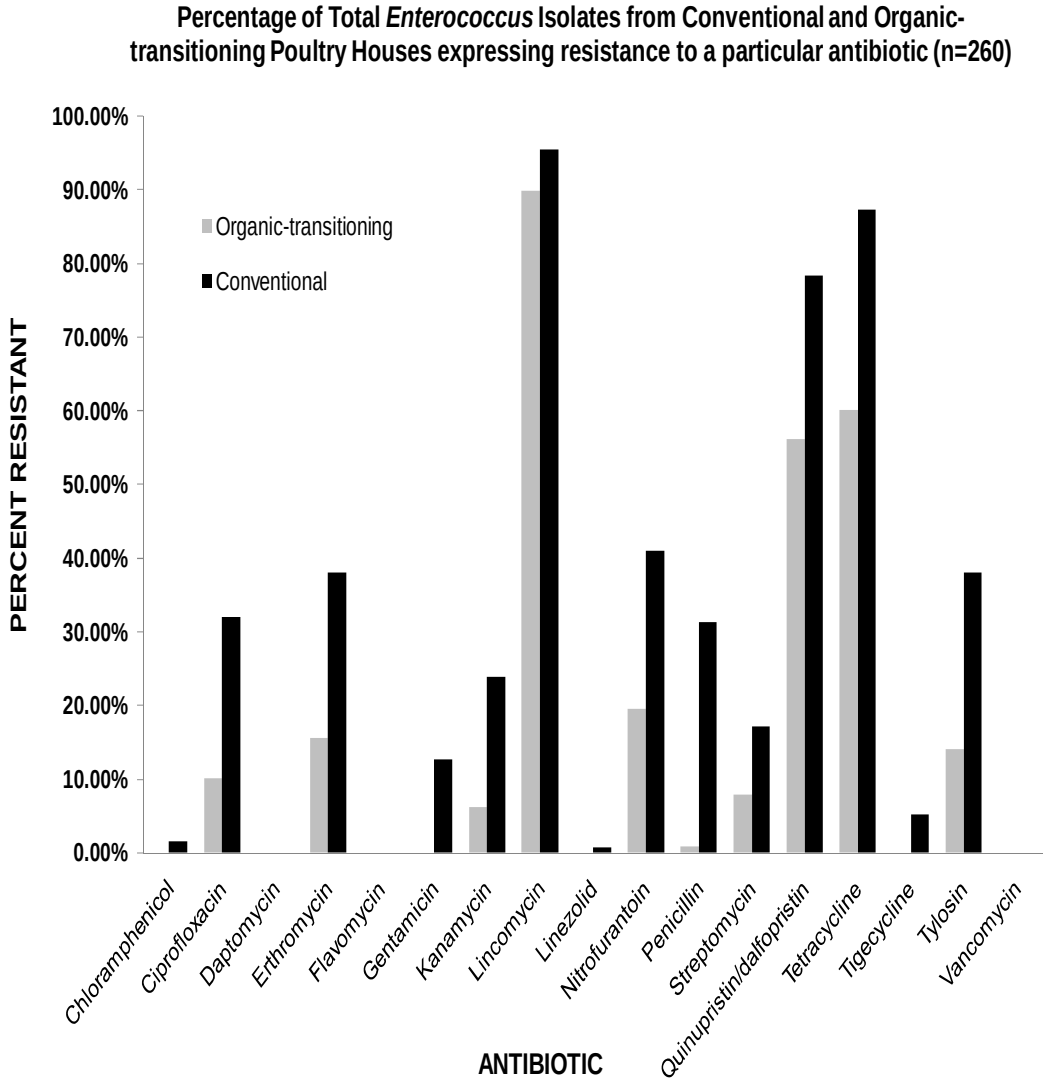


Figure 10: Percentage of Total *Enterococcus* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=260)

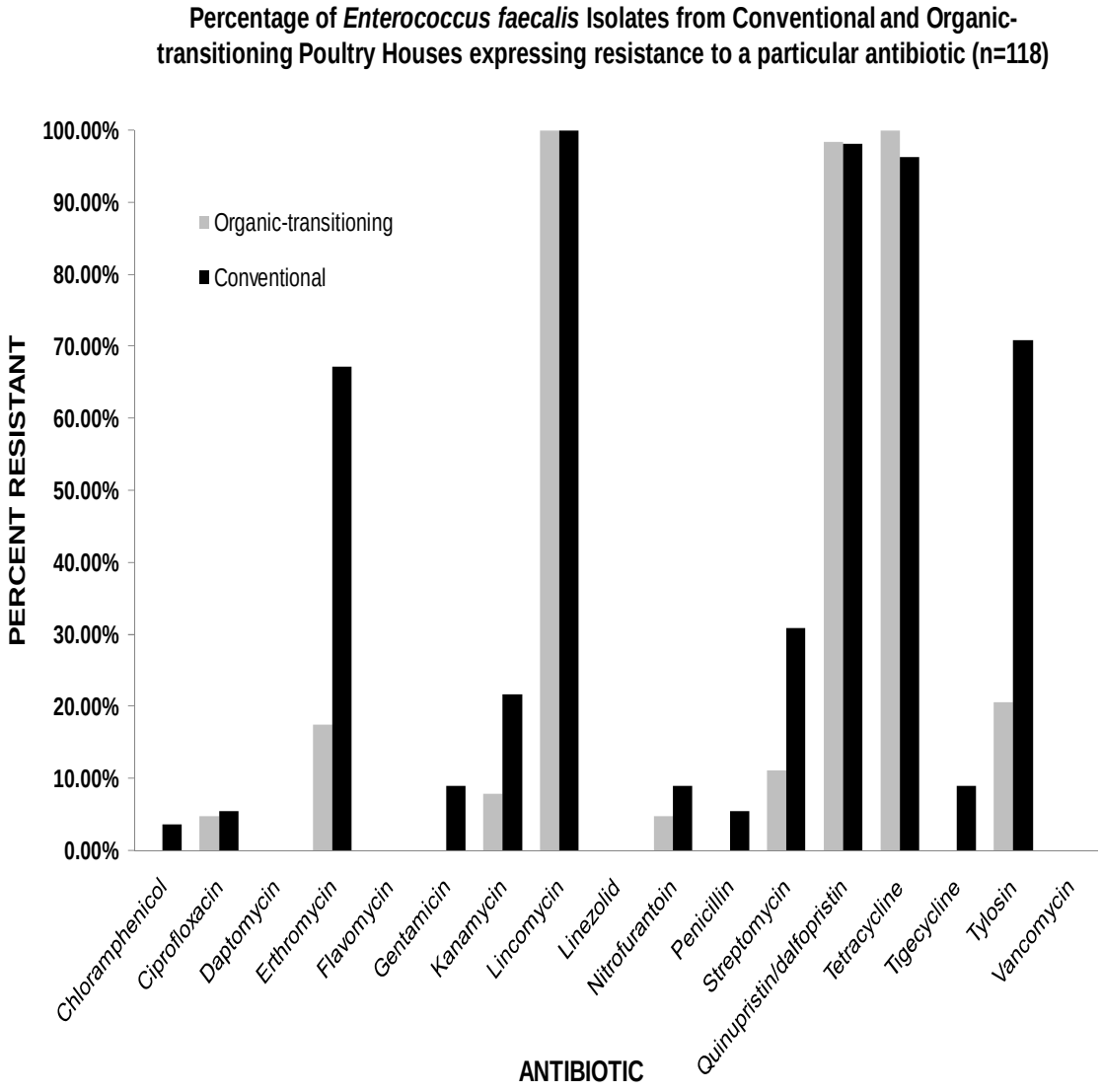


Figure 11: Percentage of *Enterococcus faecalis* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=118)

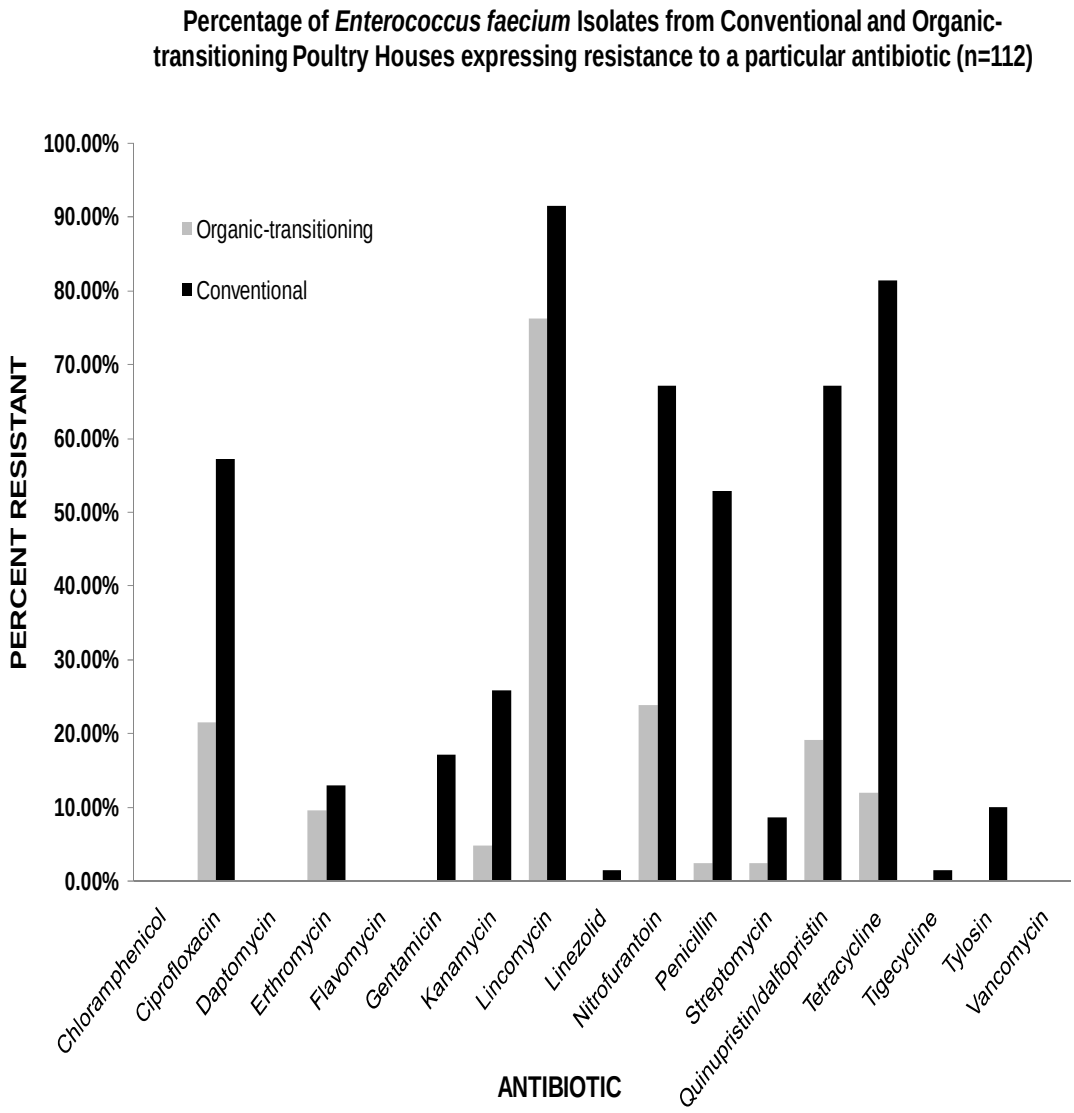


Figure 12: Percentage of *Enterococcus faecium* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=112)

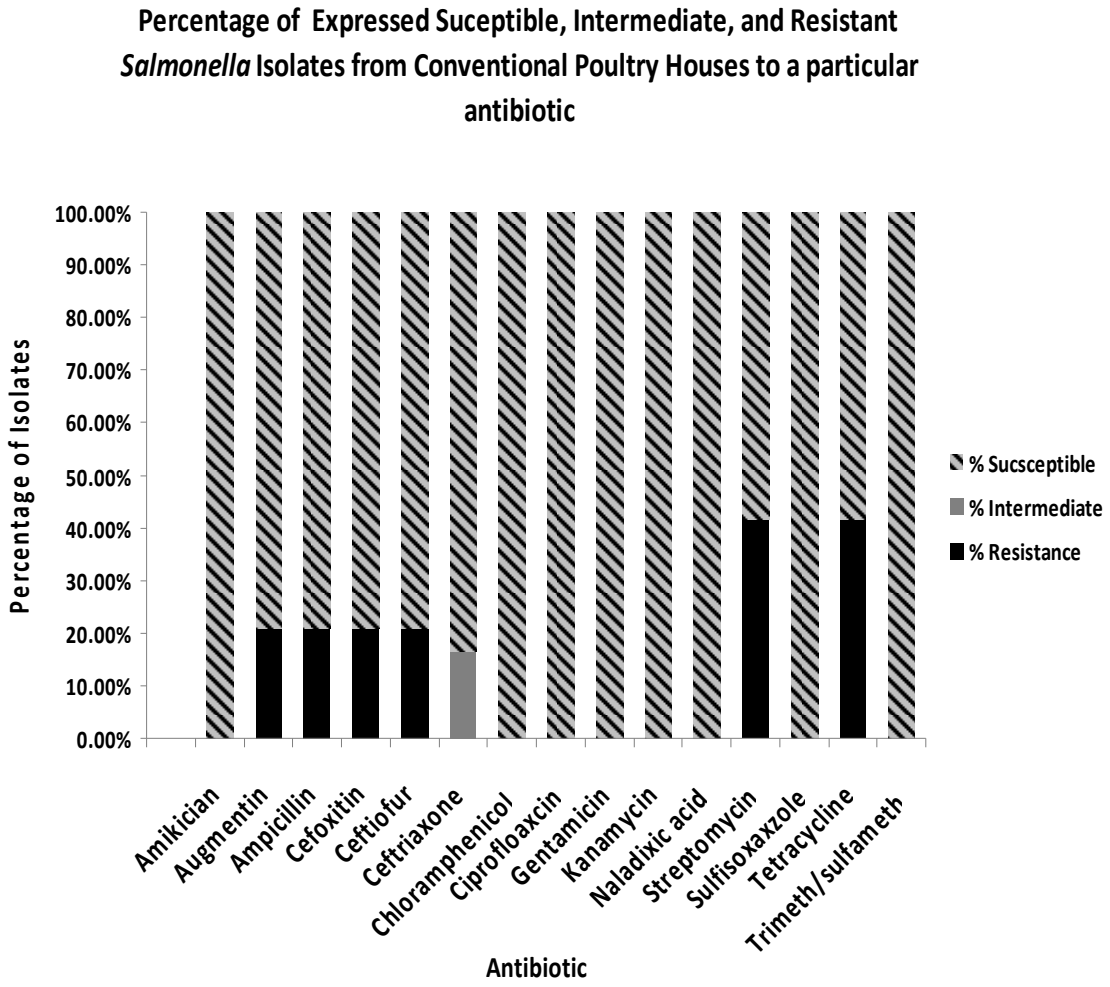


Figure 13: Percentage of Expressed Susceptible, Intermediate, and Resistant *Salmonella* spp.. Isolates from Conventional Poultry Houses to a particular antibiotic

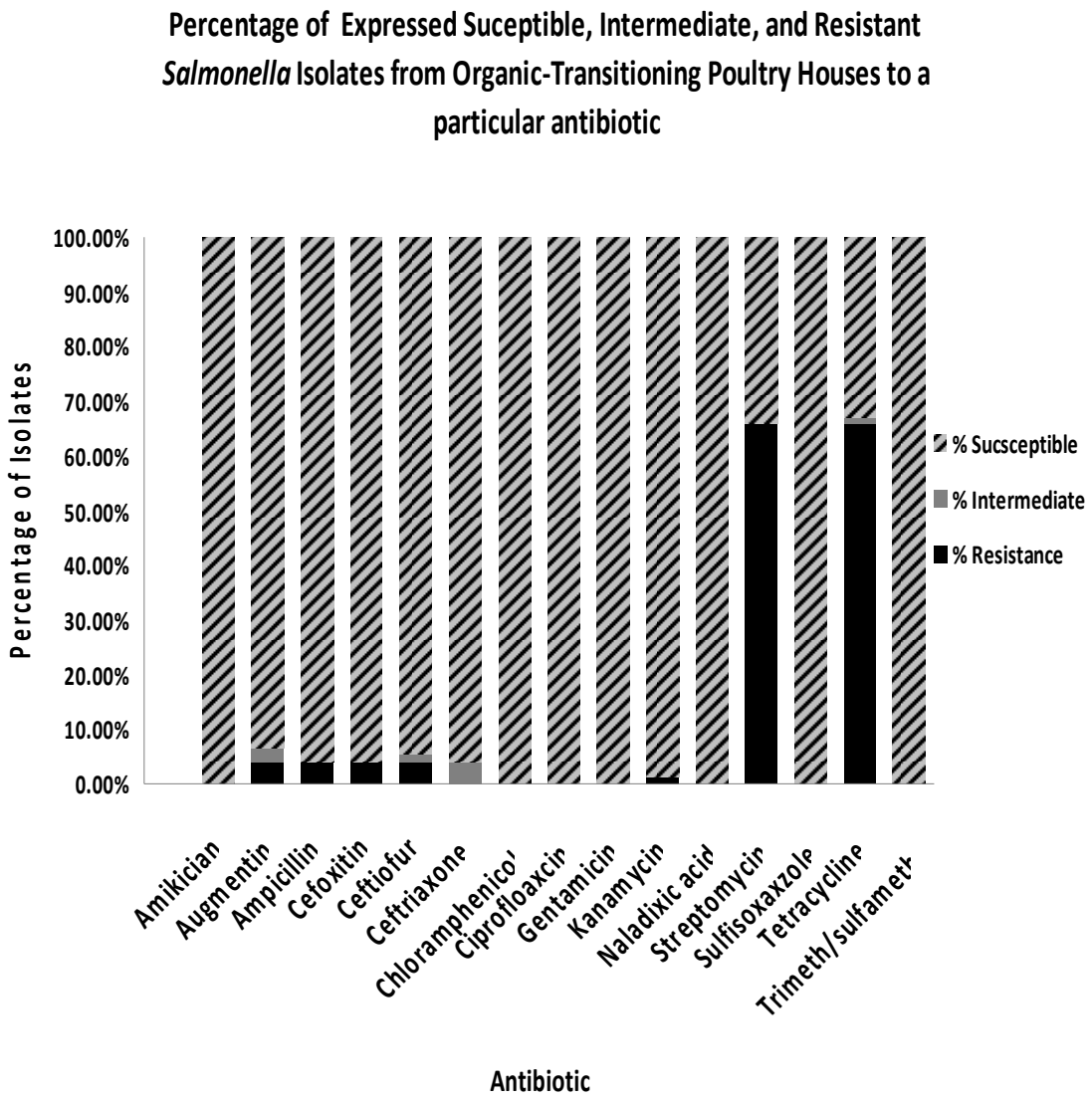


Figure 14: Percentage of Expressed Susceptible, Intermediate, and Resistant *Salmonella* spp. Isolates from Organic-Transitioning Poultry Houses to a particular antibiotic

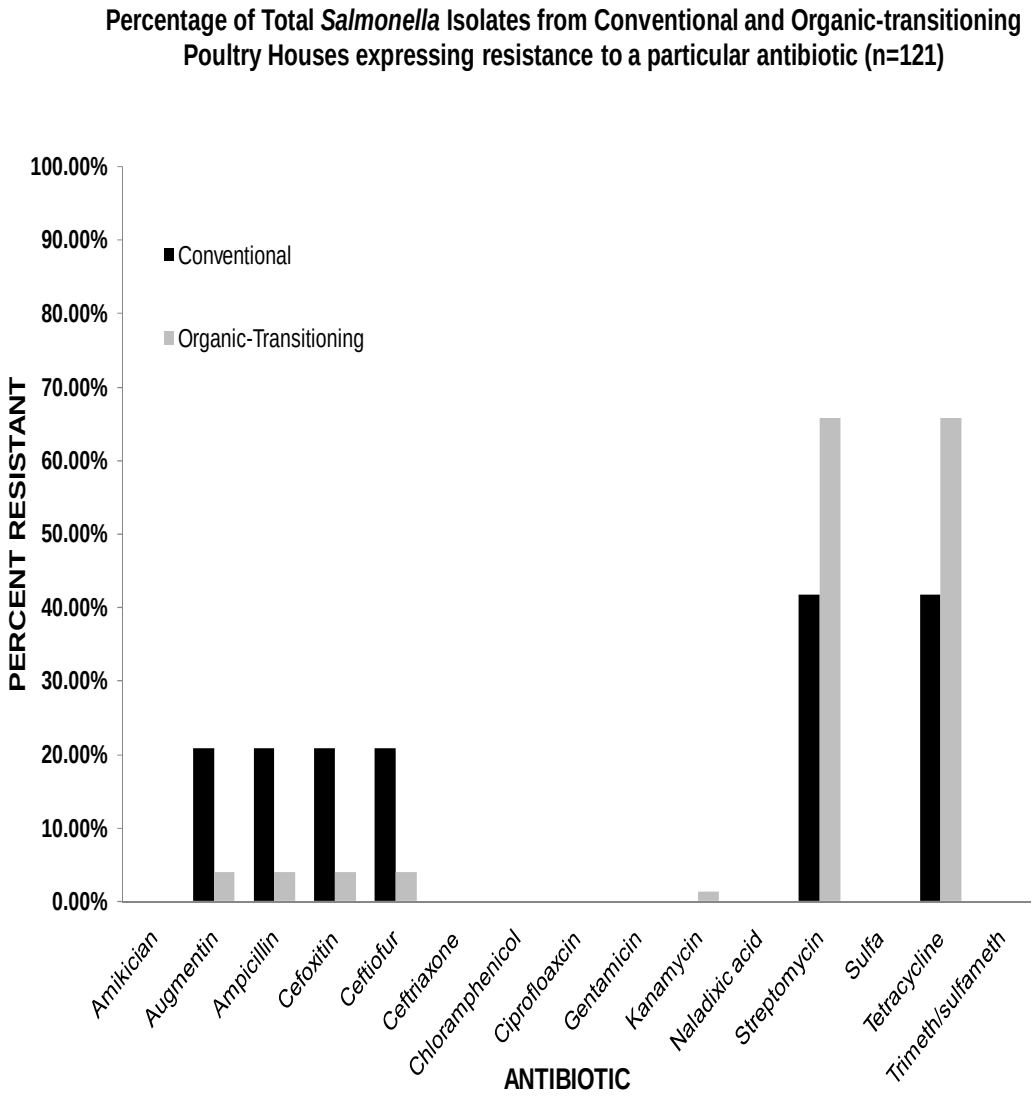


Figure 15: Percentage of Total *Salmonella* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=121)

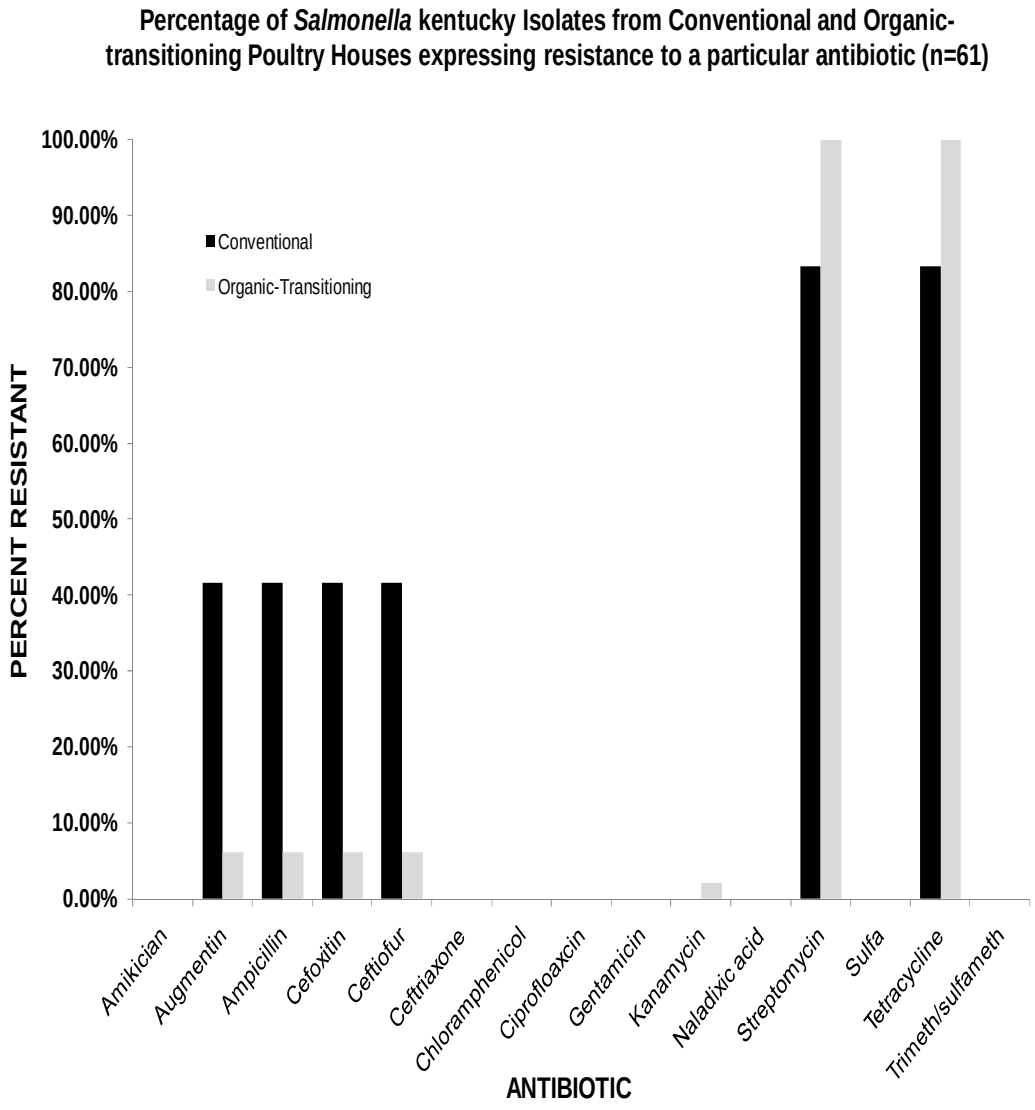


Figure 16: Percentage of *Salmonella kentucky* Isolates from Conventional and Organic-transitioning Poultry Houses expressing resistance to a particular antibiotic (n=61)

Glossary

Antibiotic: Type of antimicrobial agent made from a mold or a bacterium that kills (bactericidal), or slows the growth (bacteristatic) of other microbes specifically.

Antimicrobial resistance: Antimicrobial resistance is the result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections.

CFU: Colony-forming units. A measure of viable bacterial numbers or count.

Conventional (CONV): Poultry farm that practices standard methods used widely throughout the U.S. industry including the use of antibiotics, other antimicrobials and genetically modified organisms (GMOs) in feed

Organic (ORG): Poultry farm that undergoes strict certification process; standards apply on Day 1 of a chick's life

- No use of antibiotics, other antimicrobials or GMOs in feed
- No use of pesticides or herbicides on property
- Increased square footage per bird

Water Activity(A_w): A measurement of the equilibrium relative humidity(ERH); represents the ratio of the water pressure of sample to the water vapor pressure of pure water and reflects the active part of moisture content (unbound water) which can be exchanged between the sample and its environment.

Appendices

A. Sampling Protocol for UMD/Penn State Poultry Farm Study 2008

I. Purpose

To describe methods for the collection of samples and farm information from poultry farms that are maintaining conventional practices and from poultry farms that are transitioning to organic practices for the purpose of assessing longitudinal trends of bacterial antimicrobial resistance at these farms.

II. Scope/Limitations

This protocol applies to all poultry farms that will be included in this study, and involves the collection of meteorological data, poultry litter samples, water samples, feed samples, and additional data regarding characteristics of poultry houses, chickens, breeders and hatcheries.

III. Requirements

All personnel carrying out this protocol must obtain personal protective equipment and clothing. During sample collection, booties, coveralls, hair covers and gloves will be worn by all study personnel. Important: The accompanying "Poultry Farm Sampling Questionnaire" MUST BE FILLED OUT COMPLETELY before leaving each of the poultry houses. No abbreviations, please.

IV. Field Equipment Check List

Verify that all necessary items are present before beginning this protocol (Table 1).

V. General Terms and Definitions

a. Conventional: Refers to standard agricultural practices widespread in the industry. Can include use of pesticides, synthetic fertilizers, antibiotics and other agribusiness approaches.

b. Organic: Of or relating to foodstuff grown or raised without synthetic fertilizers or pesticides, antibiotics, chemicals or hormones.

c. Poultry Litter: A mixture of manure, feed, feathers, and the sawdust used as bedding material in poultry farms.

d. Water Activity: Water activity or A_w is the relative availability of water in a substance.

VI. Data Collection Protocols

A. General Information

In this study, 5 poultry farms that are maintaining conventional practices and 5 poultry farms that are transitioning to organic practices will be included in this study. **2** poultry houses at each of the 10 farms (if possible) will be sampled throughout the study, for a total of **20 poultry houses**. The "Poultry Farm Sampling Questionnaire" should be filled out **for each poultry house that will be sampled**. Upon arrival at each poultry house, questions 1.1. through 1.8 on the "Poultry Farm Sampling Questionnaire" should be completed as follows:

1.1 Sample Date Collection	Record as the month, day, year (e.g. 02/21/2008)
1.2 Poultry Company Name	Record the name of the poultry company associated with sampled farm
1.3 Poultry Farm Name	Record the full name of the specific poultry farm where the poultry house is located
1.4 Poultry House Code	On each farm, each poultry house that is sampled will have a unique poultry house code, i.e. PH1= poultry house 1. This same house will be sampled on all subsequent sampling trips. No two poultry houses (even if they are on different farms) will have the same poultry house code.
1.5 Poultry House Type	Record the type of poultry house being sampled: a. House transitioning to organic (intervention) group b. House maintaining conventional practices (control group)
1.6 Length of Time a Farm Has Been Organic	**(For Organic Poultry Houses Only)** Record the time in months that the sampled poultry house has been organic
1.7 Distance from Nearest Conventional Poultry House	**(For Organic Poultry Houses Only)** Record the approximate distance from the sampled organic poultry house to the nearest conventional poultry house

1.8 Other Types of Poultry Houses on Property	**(For Organic Poultry Houses Only)** Record ANY other types of poultry houses on this farm (e.g. antibiotic-free or conventional poultry houses on site)

B. Meteorological Conditions

A portable meteorological instrument will be utilized for the collection of meteorological conditions at each poultry house. This data will be recorded on the "Poultry Farm Sampling Questionnaire" in questions 2.1 through 2.5. As indicated on the "Poultry Farm Sampling Questionnaire," meteorological conditions will be collected both inside and outside of the sampled poultry houses. Prior to entering each poultry house, capture OUTSIDE meteorological conditions followed by INSIDE conditions as follows:

2.1 Ambient Temperature (OUTSIDE)	Record ambient temperature right outside of the poultry house
2.2 Relative Humidity (OUTSIDE)	Record relative humidity right outside of the poultry house
2.3 Ambient Temperature (INSIDE)	Record ambient temperature right inside of the poultry house
2.4 Relative Humidity (INSIDE)	Record relative humidity right inside of the poultry house

C. Poultry Litter Sample Collection

In each house, **3 poultry litter samples**, from the top 1 to 2 cm of the poultry litter area will be collected in ~ 500 g portions from 3 **different** locations defined by a 0.5-1.0 m^2 area. The sampled areas will be chosen at random and each sample will be collected using sterile plastic scoops and latex gloves. Fresh, plastic scoops and disposable gloves will be used to sample each new area. All poultry litter samples will be collected in sterile, sealed bags.

Sampling Identification Scheme:

(NOTE: Each sample will be given a unique sample ID that is a combination of 1) the month and year the sample was collected; 2) the poultry house code of the poultry house where it was collected; 3) the type of sample; and 4) the sample number from that poultry house. For example, a sample with this sample ID# ***03_08_PH1_L1*** *will indicate that this sample was collected in March 2008 from poultry house number*

one and this sample is the 1st poultry litter sample (L1) from this house. Water samples will be indicated with a "W" and feed samples with an "F.")

Poultry Litter Sample Collection Protocol:

Step	Procedure
1	Once inside a poultry house, chose at random 3 locations where the poultry litter samples will be collected. Each of the 3 locations should be defined by a ~0.5-1.0 m^2 area.
2	Using latex gloves and a sterile plastic scoop collect ~500 g of poultry litter from the top 1 to 2 cm of the defined ~0.5-1.0 m^2 poultry litter area.
3	Aseptically, place the sample into a sterile plastic bag and seal.
4	Label the bag with the following: the date (e.g. mm/dd/yyyy) and the Sample ID (see the description of the sampling identification scheme above), and record the Sample ID within the table in Section 3, Sample Information, of the "Poultry Farm Sampling Questionnaire."
5	Describe and record the specific location where the poultry litter sample was collected (i.e. beneath the waterers, middle of the house, corner of the house, etc.) within the table in Section 3, Sample Information, of the "Poultry Farm Sampling Questionnaire."
6	Measure the airflow (ft/min) six inches above the location where the sample was collected (direct measure from air flow meter) and record the result within the table in Section 3, Sample Information, of the "Poultry Farm Sampling Questionnaire."
7	Measure and record the water activity (Aw) at the location where the sample was collected (direct measure from PawKit water activity meter). (We need to include specific steps on how they should go about measuring Aw with the PawKit)
8	Repeat steps 1-8 for each sample. **Be sure to change plastic scoops and gloves between each sample.**

D. Water Sample and Feed Sample Collection

In addition to litter samples, water and feed samples will be collected from the poultry houses. Water samples will be collected using 500mL, sterilized polyethylene Nalgene wide-mouth environmental sampling bottles (Nalgene, Lima, OH) and feed samples will be collected using sterile plastic bags. **1 water sample** and **1 feed sample** will be collected from each poultry house on every other sampling trip. Water samples will be collected from the waterer lines and feed samples will be collected from the feed lines within the houses.

Water Sample and Feed Sample Collection Protocol:

Step	Procedure
1	During every other sample collection trip, collect **1 water sample** and **1 feed sample** from each poultry house.
2	WATER SAMPLES: Using latex gloves, collect water sample into a sterile Nalgene Bottle from the waterer in the poultry house. (i.e. nipple drinkers, cup drinkers etc.) and seal.
3	Label the bottle with the following: Date Sampled, Sample ID (e.g. 3_08_PH1_W1)
4	FEED SAMPLES: Using latex gloves and a sterile plastic scoop, collect ~250g of feed from the feed lines into a sterile plastic bag.
5	Label the bag with the following: Date Sampled, Sample ID (e.g. 3_08_PH1_F1)
6	Repeat steps 1-5 for each sample. **Be sure to change plastic scoops and gloves between each sample.**

E. Poultry House Characteristics

The following information should be filled out in Sections 4 through 7 on the "Poultry Farm Sampling Questionnaire." These data should be collected at the time of sampling and should be completed for each poultry house (i.e. There will be **one complete "Poultry Farm Sampling Questionnaire" filled out for each poultry house**).

4.1 Length of Poultry House — Measure with tape measure and record the length of the poultry house (ft.).

4.2 Width of the Poultry House — Measure with tape measure and record the width

4.3 Type of Ventilation — Record the type of ventilation inside the poultry house (i.e. tunnel, drop curtain, drop panel).

4.4 Type of Poultry Litter — Record the type of poultry litter inside the poultry house (i.e. wood shavings/ sawdust, reused poultry litter, etc).

4.5 Depth of Poultry Litter — Measure with ruler and record the depth of the poultry litter in the poultry house

4.6 Time Since Last Entire Clean-Out — Record the time since the poultry litter in

	the entire house was change (months). NOTE: This may require input from the grower)
4.7 Amount of Total Light	Measure with light meter and record the total light in the poultry house (quantitative measure).
4.8 Degree of Sunlight	Record the amount of sunlight in the poultry house (qualitative measure).
4.9 Type of Waterer	Record the type of waterer inside the poultry house (i.e. nipple, cup drinker, and trough).

F. Poultry Farm Characteristics, Chicken Characteristics, Breeder Characteristics, and Hatchery Characteristics: <u>Interview with Poultry Grower</u>

PLEASE NOTE: ****This portion of the protocol will entail an <u>IN-PERSON</u> interview with each grower on each of the sampled farms. The following questions will be asked of the poultry grower in order that the remainder of the "Poultry Farm Questionnaire" can be completed.

<u>INSTRUCTIONS: Go directly to Questions 4.9-7.3 on the "Poultry Farm Sampling Questionnaire" for pre-written questions to be administered in person to the grower on each sampled poultry farm.</u>

G. Ensure that the "Poultry Farm Sampling Questionnaire" is Complete

IMPORTANT: PLEASE DO NOT LEAVE THE POULTRY HOUSE UNTIL EVERY FIELD OF THE "POULTRY FARM SAMPLING QUESTIONNAIRE" HAS BEEN COMPLETED. FAILURE TO FILL OUT A QUESTIONNAIRE COMPLETELY FOR EACH POULTRY HOUSE WILL COMPROMISE THE STUDY RESULTS.

H. Make a copy of the "Poultry Farm Sampling Questionnaire" for your records and send the original questionnaire, along with the environmental samples, to UMD at the following address:

Amy R. Sapkota
UMCP School of Public Health
Maryland Institute for Applied Environmental Health
2308 HHP Bldg
College Park, MD 20742

B. Poultry Farm Questionnaire

Poultry Farm Sampling Questionnaire
UMD/Penn State Poultry Farm Study 2008

1. **General Information**

1.1 Sample collection date *(mm/dd/yyyy)*_____

1.2 What is the name of the poultry company? *(please specify)*_____

1.3 What is the name of the specific poultry farm? *(please specify)*_____

1.4 What is the poultry house code? _____ (*On each farm, we will assign each poultry house that we sample a unique poultry house code, such as PH1 for poultry house 1. This same house will be sampled on all subsequent sampling trips*.)

1.5 In what year was the poultry house built? _____

1.6 What is the type of poultry house? *(Circle one)*

a) House transitioning to **organic** (intervention group) **[If it is this type of poultry house, go to question 1.7]**

b) House maintaining **conventional** practices (control group) **[If it is this type of poultry house, SKIP to question 2]**

1.7 How long has this poultry house been an **organic** house? _____months

1.8 What is the approximate distance from this **organic** poultry house to the **nearest conventional** poultry house? *(Circle one)*

a) < ½ mile
b) ½ mile to 1 mile
c) 2 to 5 miles
d) 6 to 10 miles
e) >10 miles

1.9 Are there other types of poultry houses on this farm? *(Circle one)*

f) Yes, there are also antibiotic-free poultry houses on this farm
g) Yes, there are also conventional poultry houses on this farm
h) No

2. **Meteorological Conditions** *(To be measured with portable meteorological instrument)*

2.1 What is the ambient temperature right **outside** of the poultry house? ____°F

2.2 What is the relative humidity right **outside** of the poultry house? _____%

2.3 What is the ambient temperature **inside** of the poultry house? _____°F

2.4 What is the relative humidity **inside** of the poultry house? _____%

2.5 Was it raining when the samples were collected? *(Circle one)* Yes No

2.6 What were the cloud/sun conditions at the time samples were collected? *(Circle one)*

a) Clear and sunny (Free from clouds, fog, mist or dust haze)
b) Mostly sunny (Little chance of the sun being obscured by clouds)
c) Partly cloudy (Predominantly more clouds than clear sky)
d) Overcast with complete cloud cover (Sky completely covered with clouds)

3. Sample Information

(NOTE: Each sample will be given a unique sample ID that is a combination of 1) the month, day and year the sample was collected; 2) the poultry house code of the poultry house where it was collected; 3) the type of sample; and 4) the sample number from that poultry house. For example, a sample with this sample ID# ***03_31_08_PH1_L1*** *will indicate that this sample was collected on March 31, 2008 from poultry house number one and this sample is the 1st poultry litter sample (L1) from this house. Water samples will be indicated with a "W" and feed samples with an "F.")*

Sample ID#	**Sample Type**	**Where was the sample collected within the poultry house?** *(ie. beneath the drinkers; in the middle of the house; from the water lines etc.)*	**What was the airflow (ft/min) *six inches* above the location where the sample was collected?** (*Direct reading from airflow meter)*	**What was the water activity (Aw) at the location where the sample was collected?** *(Direct reading from water activity meter)*	**What was the amount of light (lux) *12 inches* above the location where the sample was collected?** *(Direct reading from light meter)*	
					Fans ON	**Fans OFF**
	Litter	Under feeder				
	Litter	Under waterer				
	Litter	Middle of house				
	Water	Source (or source after primary treatment)	Not applicable	Not applicable	Not applicable	Not applicable
	Water	End of line	Not applicable	Not applicable	Not applicable	Not applicable
	Feed	Hopper in house	Not applicable	Not applicable	Not applicable	Not applicable
	Soil*	Outside	Not applicable	Not applicable	Not applicable	Not applicable
	Booties	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

*NOTE: At organic farms, a soil samples will be collected from the area where the chickens are allowed outdoors. At the conventional farms, a soil sample will be collected from an area where poultry is land-applied if possible.

4. Poultry House Characteristics

4.1 **What is the **length** of the poultry house? _____feet

4.2 **What is the **width** of the poultry house? _____feet

4.3 What type of **ventilation** system is in use inside the poultry house? *(Circle one)*

a) Tunnel ventilation
b) Drop curtain
c) Mechanically ventilated
d) Other *(please specify)*_____

4.4 What was the type of **poultry litter** in the poultry house at the time of sampling? *(Circle one)*

a) Wood shavings/sawdust
b) Reused poultry litter/Build-up
c) Peanut hulls
d) Rice hulls
e) Other *(please specify)*_____

4.5 What is the **depth** of the poultry litter at the poultry litter sampling location that was away from both the drinkers and the feed lines? ____ inches

4.6 **How long ago was the poultry litter in the **entire** house changed? _____months

4.7 How much **sunlight** was in the poultry house at the time of sampling? *(Circle one)*

a) A lot of sunlight
b) Some sunlight
c) Not a lot of sunlight
d) Very little sunlight
e) No sunlight

4.8 What is the type of **drinker** in the poultry house? *(Circle one)*

a) Nipple drinkers
b) Cup drinkers
c) Bell drinkers
c) Other

4.9 What is the design of the **drinker system** in the poultry house? *(Please specify)*_____

4.10 What is the design of the **feed system** in the poultry house? *(Please specify)*_____

NOTE: You will need to conduct an interview with each poultry grower to answer the following questions.

It is possible, that the grower (particularly the conventional growers) will not have answers for the following questions: 4.13, 4.14, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, and 6.7. If this is the case, we will have to ask these questions of the poultry company.

4.11 **What is the source of water for the poultry house?

a) Well water
b) Public water supply
c) Other *(please specify)*_____

4.12 **What company supplies the poultry feed? *(please specify)*

_____

4.13 **What type of feed was used in the poultry house at the time of sampling?

a) Broiler Starter
b) Broiler Grower/Finisher
c) Broiler Grower Concentrate
d) Other *(please specify)*_____

4.14 **Were **antibiotics/antimicrobials** used in the poultry **feed** for the current flock? *(Circle one)*

Yes No

4.15 **If antibiotics/antimicrobials were used in the poultry feed, what specific antibiotics/antimicrobials were used for this flock **at any time** before or during sampling? *(Circle all that apply)* **NOTE: Most likely, the growers will not know this information, so <u>we will need to obtain it from the company.</u>**

a) No antibiotics/antimicrobials were ever used in the poultry feed of this flock
b) Bambermycin
c) Bacitracin
d) Chlortetracycline
e) Oleandomycin
f) Penicillin
g) Tylosin
h) Tetracycline
i) Virginiamycin
j) Lincomycin
k) Arsanilic acid
l) Roxarsone
m) Carbarsone
n) Salinomycin
o) Lasalocid
p) Narasin
q) Monensin
r) Other *(please specify)*_____
s) Other *(please specify)*_____
t) Other *(please specify)*_____

4.16 **Were **antibiotics/antimicrobials** used in the **water** for the current flock? *(Circle One)* Yes No

4.17 **If antibiotics/antimicrobials were used in the poultry water, what specific antibiotics were used for this flock **at any time** before or during sampling? **NOTE: The grower will have this information.**

a) No antibiotics/antimicrobials were used in the water for this flock

b) Bacitracin
c) Chlortetracycline
d) Tylosin
e) Fluoroquinolone
f) Other *(please specify)*_____
g) Other *(please specify)*_____
h) Other *(please specify)*_____

4.18 **Were any other feed or water additives used for the current flock? *(Circle one)*
Yes No

4.19 If any other feed or water additives were used for the current flock, what specific additives were used? *(Circle all that apply)*
a) No other feed or water additives were used for the current flock
b) Citric acid (in water)
c) Vitamin D (in water)
d) PWT (pH amendment)
e) Acidified Cu (copper) sulfate
f) Other *(please specify)*_____

5. Chicken Characteristics

5.1 **What was the number of chickens introduced with the current flock? _____ chickens

5.2 **What was the strain of the current flock?
a) Ross
b) Ross Cobb
c) Cobb/Cobb
d) Mixture *(Please specify)*_____
_____
e) Other *(please specify)*_____

5.3 **What was the age (in days) of the flock at the time of sampling? _____ days

5.4 **What was the date that the current flock arrived at the farm (ie. the "date in")

(mm/dd/yyyy)_____

5.5 **What was the mortality rate (%) of the current flock at the time of sampling? _____%

5.6 **What is the average amount of time (minutes) the current flock spends outdoors each day?_____ min

6. Hatchery Characteristics (NOTE: We may have to ask the **company** for the following information)

6.1 **What is the name of the hatchery where the current flock came from? *(please specify)*

_____

6.2 **Does this hatchery use **antibiotics/antimicrobials** for any purpose? *(Circle one)*
Yes No

6.3 **If the hatchery **does** use **antibiotics/antimicrobials** for any purpose, what specific compounds are
used?

a) No antibiotics/antimicrobials were used at the hatchery
b) Gentamicin
c) Naxcel (Cephalosporin)
d) Other *(please specify)*_____
e) Other *(please specify)*_____
f) Other *(please specify)*_____

6.4 **Does the hatchery use **vaccinations** for any purpose? *(Circle one)* Yes No

6.5 **If the hatchery **does** use **vaccinations**, what specific vaccinations are used? *(Circle all that apply)*

a) No vaccinations are used
b) Coccivac
c) Merrick's
d) Newcastle
e) Bronchitis
f) HVT/SB1
g) IBD
h) N/B New Hatch
i) Other *(please specify)*_____

6.6 **Does the hatchery use **probiotics** for any purpose? *(Circle one)* Yes No

6.7 **If the hatchery **does** use **probiotics,** what are the specific compounds that are used? *(Circle all that apply)*

a) No probiotics are used
b) Avacor
c) Other *(please specify)*_____

7. Breeder Characteristics (NOTE: We may have to ask the **company** for the following information)

7.1 **What is the name of the breeder(s) where the current flock came from? *(please specify)*

_____

7.2 **Does this breeder(s) use **antibiotics/antimicrobials** for any purpose? *(Circle one)*
Yes No

7.3 **If the breeder(s) **does** use **antibiotics/antimicrobials** for any purpose, what specific compounds are
used?

a) No antibiotics/antimicrobials were ever used in the poultry feed of this flock

b) Bambermycin
c) Bacitracin
d) Chlortetracycline
e) Oleandomycin
f) Penicillin
g) Tylosin
h) Tetracycline
i) Virginiamycin
j) Lincomycin
k) Arsanilic acid
l) Roxarsone
m) Carbarsone
n) Salinomycin
o) Lasalocid
p) Narasin
q) Monensin
r) Other *(please specify)*_____
s) Other *(please specify)*_____
t) Other *(please specify)*_____

7.4 **Does the breeder(s) use **vaccinations** for any purpose? *(Circle one)* Yes No

7.5 **If the breeder(s) **does** use **vaccinations**, what specific vaccinations are used? *(Circle all that apply)*

a) No vaccinations are used
b) Coccivac
c) Merrick's
d) Newcastle
e) Bronchitis
f) HVT/SB1
g) IBD
h) N/B New Hatch
i) Wormer
j) Rheovirus
k) Other *(please specify)*_____

7.6 **Does the breeder(s) use **probiotics** for any purpose? *(Circle one)* Yes No

7.7 **If the breeder(s) **does** use **probiotics,** what are the specific compounds that are used? *(Circle all that apply)*

a) No probiotics are used
b) Avacor
c) Other *(please specify)*_____

C. *Enterococcus* Protocol (Isolation from Poultry Litter and Poultry Feed)

Objective: Enrichment experiment for isolating, purifying, and archiving *Enterococcus* derived from poultry litter and feed samples using Enterococcosel Broth (Difco), Enterococcosel Agar and BHI Agar.

Pre Sample Arrival(Week Before)

1. Calculate the amount of Broth and Agar needed for sample processing.
2. Prepare Enterococcosel Broth
    a. Suspend 43 g of the powder in a 1 L of d H20
    b. Mix thoroughly , heat and boil for 1 min to completely dissolve the powder.
    c. Autoclave at 121C for 30 min
    d. Cool to 50 °C
    e. Place in the refrigerator at 4°C for later use.

3. Prepare Enterococcosel Agar
    a. Suspend 56 g of powder in 1 L of dH20.
    b. Mix thoroughly , heat and boil for 1 min to completely dissolve the powder.
    c. Autoclave at 121C for 30 min
    d. Cool to 50 °C
    e. Pour into 100 x 15mm Petri dishes and store.
    f. Place in the refrigerator at 4°C for later use.

4. Prepare BHI Agar
    a. Suspend 52 g of powder in 1 L of dH20.
    b. Mix thoroughly, heat and boil for 1 min to completely dissolve the powder.
    c. Autoclave at 121C for 30 min
    d. Cool to 50 °C
    e. Pour into 100 x 15mm Petri dishes and store.
    f. Place in the refrigerator at 4°C for later use.

Day 1: **Sample Arrival and Enrichment**

1. Label all sample containers (133 mL) with the appropriate poultry house, sample media code, i.e. PH1_LI, etc.
2. Aseptically weigh and add 10 grams of poultry litter/feed into 133 mL sample containers. Under the BSC, aseptically add 100mL of Enterococcosel Broth to each 133mL sample container.
3. Swirl gently to evenly distribute the Enterococcosel Broth among the sample.
4. Place the container into the incubator overnight (24 hr) at 41°C.
5. Set up a positive (+) and negative (-) control broth.

Day 2: **Isolation**

Today you will streak your enrichment culture for isolation on Enterococcosel agar (EA) media. EA has nutrients appropriate for the growth of enterococci and will presumptively select for enterococci. Additionally, this media contains bile esculin and sodium azide, and therefore, in the presence of enterococci species, a brown-black precipitate will be visible beneath the presumptive colony in the agar.

Obtain your poultry litter/feed enrichment culture from the incubator and obtain an EA plate.

1. Label your EA plate (Initials, Date, PH1_L1,ENT).
2. Take a 10ul loopful of your enrichment culture and streak your plate for isolation of *Enterococcus.* Incubate overnight at 41 °C.
3. Streak a (+) control and (-) control plate and incubate overnight at 41 °C.

Day 3: **Purification**

Target colony: Very small, Light→Dark brown colonies with black precipitate; Take 3 target organisms from each sample and streak for purification onto BHI.

Today you will streak your enterococci culture for purification on BHI agar media.
Obtain your EA plate from the incubator and record results (i.e. presence of absence of typical enterococci growth)

1. Label your BHI plate (Initials, Date, PH1_L1,ENT).
2. Select 3 target colonies and streak each colony onto your BHI plate for purification of each *Enterococcus* isolate. Incubate overnight at 41 °C.
3. Streak a (+) control and (-) control plate and incubate overnight at 41°C.

Day 4: **Biochemical Testing**

Today you will do a Gram Stain, catalase test and PYR test to presumptively identify *Enterococcus* from your positive poultry litter/feed samples.

- The Gram stain will confirm that you have a pure culture and it will also confirm that you have a Gram positive coccus (morphology and gram reaction for *Enterococcus*).
- The PYR test is a rapid, colorimetric test recommended for use in qualitative procedures for the detection of pyrrolidonyl arylamidase activity for presumptive identification of enterococcoci, group A streptococcoci, and Escherichia coli.
- The catalase test examines the ability to breakdown hydrogen peroxide by catalase. Those organisms possessing the catalase enzyme will break down hydrogen peroxide into water and oxygen. The oxygen causes bubbles to form within, seconds, indicating a positive test. The absence of bubbles is considered a negative test. *Enterococcus* is catalase negative (or very weakly positive).

Obtain your BHI purification plates from the incubator and record results. Make sure that you have a pure (and NOT mixed) culture.

1. Gram Stain
    a. Perform Gram Stain as directed
    b. Record observations

2. PYR Test
    a. Test isolates should be 18-24 hours old and taken from non-selective media, such as BHI
    b. Using forceps, place the disk on a clean microscope slide or in the lid of a Petri dish free from excess moisture.
    c. Moisten the disk slightly with 5-10 ul of demineralized water using a micropipette or a 10 uL inoculating loop. DO NOT OVERSATURATE.
    d. Remove a visible "paste" of the test isolate using a sterile loop.
    e. Rub the inoculums gently into a small area of the disk.
    f. Add one (1) drop of PYR Reagent to the disk.
    g. Allow up to one minute for a color change.
        i. Positive test= pink to red color development w/in 1 min of applying PYR reagent
        ii. Negative test= Cream, yellow, or no color change within one minute of applying PYR Reagent

3. Catalase Test
    a. Collect an empty Petri dish and place one drop of 3 % hydrogen peroxide/per sample on to surface of Petri dish
    b. Take small swab from each sample and place into the 3 % hydrogen peroxide.
    c. Examine plates for bubbles. Presence of bubbles= positive result; Absence of bubbles =negative result
    d. Record observations

IMPORTANT: If you have Black precipitate, (+) gram stain, (+) PYR test, and (-) (or very weakly positive) catalase test, then archive the isolate as follows:

Day 4 or 5: **Archiving of Sample Isolates**

Today you will archive *Enterococcus* isolates from your BHI purification plates.

Obtain your BHI purification plates from the incubator. Also obtain Brucella Broth w/ 15% glycerol

1. Observe and record the results of your BHI plate. Compare your plate to the control plate and make sure that you have a pure (and NOT mixed) culture.
2. Label your Brucella Broth w/ 15% glycerol tube with the following information: (Date of sampling, PH_L1_E1… E2…E3…(Each isolate will have a continuous number independent of the poultry house label).
3. Using a sterile swab, collect a substantial amount of enterococci. Place into the Brucella Broth and gently swirl in order to get remainder off of cotton swab.

4. On laboratory data sheet, record information about isolate including location in the freezer. Place Enterococci isolate in the -80 freezer.

D. *Salmonella* Protocol (Isolation from Poultry Litter and Poultry Feed)

Objective: Enrichment experiment for the isolation, purification, and archiving of *Salmonella* derived from poultry litter and feed samples using Lactose Broth, TT Hajna Broth (Difco), XLT4 agar and BHI agar.

PreSample Arrival(Week Before)

5. Calculate the amount of Broth and Agar needed for sample processing.
6. Prepare Lactose Broth
    a. Suspend 13 g of the powder in 1 L of d H20
    b. Mix thoroughly, heat and boil for 1 minute to completely dissolve the powder.
    c. Autoclave at 121C for 30 min
    d. Cool to 50 C
    e. Place in the refrigerator at 4C for later use.
7. Prepare TT Hajna Broth
    a. Suspend 91 g of the powder in a 1 L of d H20
    b. Mix thoroughly, heat and boil for 1 minute to completely dissolve the powder.
    c. Cool to 50 C in waterbath
    d. Place in the refrigerator at 4C for later use.

8. Iodine Solution
    a. 40 mL iodine Solution
        i. 5 g of iodine crystals and 8 g of potassium iodide dissolved in 40 mL dH20
        ii. Store in bottle wrapped in aluminum foil at 4C

9. Prepare XLT4 Agar
    a. Suspend 59 g of powder in 1 L of dH20.
    b. Add 4.6 mL of XLT4 Agar Supplement (is the supplement added after the boiling step?)
    c. Mix thoroughly, heat and boil for 1 minute to completely dissolve the powder. (total time 20-25 minutes)
    d. DO NOT AUTOCLAVE
    e. Cool to 50 C
    f. Pour into 100 x 15mm Petri dishes and store.
    g. Place in the refrigerator at 4°C for later use.

10. Prepare BHI Agar
    a. Suspend 52 g of powder in 1 L of dH20.
    b. Mix thoroughly, heat and boil for 1 min to completely dissolve the powder.

c. Autoclave at 121C for 30 min
d. Cool to 50 °C
e. Pour into 100 x 15mm Petri dishes and store.
f. Place in the refrigerator at 4°C for later use.

11. Triple Sugar Iron Agar (TSI)
    a. Suspend 59.4 g of powder in 1 L of dH20.
    b. Mix thoroughly, heat and boil for 1 min to completely dissolve the powder.
    c. Sterilize by autoclaving at not over 118C for 15 min
    d. Cool in a slanted position such that deep butts are formed
    e. Place in the refrigerator at 4°C for later use.

12. Lysine Iron Agar (LIA)
    a. Suspend 34.5 g of powder in 1 L of dH20.
    b. Mix thoroughly, heat and boil for 1 min to completely dissolve the powder.
    c. Autoclave at 121C for 12 min
    d. Cool in a slanted position such that deep butts are formed (at least 4cm)
    e. Place in the refrigerator at 4°C for later use.

Day 1: **Sample Arrival and Pre-Enrichment**

6. Label all sample containers (133 mL) with the appropriate poultry house, sample media ID, i.e. PH1_LI, etc.
7. Aseptically weigh and add 10 grams of poultry litter/feed into 133 mL sample containers. Under the BSC, aseptically add 100mL of Lactose Broth to each 133 sample container.
8. Swirl gently to evenly distribute the Lactose Broth among the sample.
9. Place the container into the incubator overnight (24 hr) at 37°C.
10. Set up a positive (+) and negative (-) control broth

Day 2: **Enrichment**

Today you will perform the enrichment step for *Salmonella*

1. Obtain your poultry litter/feed *Salmonella* inoculums from the incubator and obtain sterile 133mL sample container cups.
2. Label your sample container (Initials, Date, PH1_L1 SAL)
3. Add iodine solution (1.2 mL per 15mL of Hajna)
4. From the Lactose Broth suspension, add an aliquot (1mL) of the suspension to 15 mL Hajna Tetrathionate broth (make sure to add iodine solution).
5. Incubate overnight at 37C
6. 2nd Enrichment: Leave TT Hajna enrichments on bench for 4 nights (if samples are initially negative, these secondary enrichments will be used to double check the status of the samples)

Day 3: **Isolation**

Today you will streak your *Salmonella* culture for isolation on XLT4 agar media. XLT4 has nutrients appropriate for the growth of *Salmonella* and will presumptively select for *Salmonella*. If *Salmonella* is present, the media will turn a yellow color and the colonies will appear either completely black or yellow-ish with a black center.

Obtain your poultry litter/feed enrichment culture from the incubator and obtain an XLT4 plate.

4. Label your XLT4 plate.(Initials, Date, PH1_L1,SAL)
5. Take a 10ul loopful of your enrichment culture and streak your plate for isolation of *Salmonella.* Incubate overnight at 37 °C.
6. Streak a positive and negative control plate and incubate overnight at 37C.

Day 4: **Purification**

Target colony: Black colonies associated with a color change (to yellow) on XLT4 agar. If positive, Take 3 target organisms from each sample and streak onto BHI. If other samples are negative, take 10 colonies from the positive samples. On samples without target organisms, return to step 6 under Day 2: 2nd Enrichment and restreak from TT Hajna 5 days after the initial enrichment. Place plates back into the 37C incubator and check after 24-48 hours.

Today you will streak your isolated colonies for purification on BHI agar.
Obtain your XLT4 plates from the incubator and record results of the isolation step (i.e. Presence or absence of typical *Salmonella* growth).

4. Label your BHI plate (Initials, Date, PH1_L1,ENT).
5. Select 3 to 10 isolated target colonies and streak each colony for purification on a BHI plate. Incubate overnight at 37 °C.
6. Streak a positive control plate and a negative control plate and incubate overnight at 37C.

Day 4: **Biochemical Testing**

Today you will do LIA and TSI agar slant tests to presumptively identify the *Salmonella* isolates from each of your poultry litter/feed sample.

- The TSI agar slant test examines the ability of a microorganism to ferment sugars and to utilize iron to produce hydrogen sulfide. Presumptive (+) cultures have alkaline (red) slants and acid (yellow) butts, with or without H2S production (blackened agar). Do not exclude H2S negative slants.

- The LIA agar slant test examines the microorganisms' ability for lysine decarboxylantion, lysine deamination(formation of red-colored products at the top of medium) and hydrogen sulfide production (black precipitate).LIA: Presumptive (+) cultures have an alkaline (purple) slants and alkaline(purple) butts. Consider only a distinct yellow coloration in the butt as an acid (negative) reaction. *** Do not

eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Some non- *Salmonella* cultures produce a brick-red reaction in LIA slants.

- Regardless of TSI reaction, all cultures that give an alkaline butt in LIA should be retained as presumptive *Salmonella* isolate. Cultures that give an acid butt in LIA and an alkaline slant & acid butt in TSI should be retained as potential *Salmonella* isolates. Cultures with an acid butt in LIA, acid slant & acid butt in TSI should be discarded as non-*Salmonella*.

Obtain your BHI plates from the incubator and record results.

4. TSI and LIA agar slant test
    a. With sterile inoculating loop, lightly touch the center of a chosen colony.
    b. Inoculate TSI slant by streaking slant and stabbing butt.
    c. Without flaming, inoculate LIA slant by stabbing but twice (2) and then streaking slant. LIA slants must have a deep butt (4cm).
    d. Incubate TSI and LIA slants at 35°C for 24 ± 2 h.
    e. Loosely cap tubes to maintain aerobic conditions while incubating slants for the prevention of excessive H_2S production

IMPORTANT: If you have Black colonies with yellow agar color change on XLT4 agar, (+) LIA agar slant, (+) TSI agar slant (or the exceptions noted above) then archive the isolates as follows:

Day 5 or 6: **Archiving of Sample Isolates**

Today you will archive your purified isolates that are currently on BHI plates.

Obtain your BHI plates from the incubator. Also obtain Brucella Broth w/ 15% glycerol

5. Observe and record the results of your BHI plate. Compare your plate to the control plate and make sure that you have a pure (and NOT mixed) culture.
6. Label your Brucella Broth w/ 15% glycerol tube with the following information: (Date of sampling, PH_L1_SAL1… SAL2…SAL3… (Each isolate will have a continuous number independent of the poultry house label).
7. Using a sterile swab, collect one *Salmonella* colony from each purification. Place into the Brucella Broth w/ 15% glycerol and gently swirl in order to get remainder off of cotton swab.
8. On laboratory data sheet, record information about isolate including location in the freezer. Place *Salmonella* isolate in the -80 freezer.

Reference List

Nosocomial enterococci resistant to vancomycin--United States, 1989-1993 (1993). *MMWR Morb.Mortal.Wkly.Rep., 42*, 597-599.

Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food--10 States, 2008 (2009). *MMWR Morb.Mortal.Wkly.Rep., 58*, 333-337.

Aarestrup, F. M. l. (1999). Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *International Journal of Antimicrobial Agents, 12*, 279-285.

Agricultural Marketing Resource Center. (2008). Commidity Poultry. Retrieved January, 28, 2009, from http://www.agmrc.org/commodities__products/livestock/poultry/commodity_poultry.cfm

Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. Campylobacter jejuni--an emerging foodborne pathogen. Emerg.Infect.Dis. 5:28-35.

Andrews FW, Horder TJ. A study of the streptococcus pathogenic for man. Lancet 1906; 2 : 708. Andrewes, F. W., and T. J. Horder. 1906. A study of the streptococci pathogenic for man. Lancet ii:708-713.

Angkititrakul, S., Chomvarin, C., Chaita, T., Kanistanon, K., & Waethewutajarn, S. (2005). Epidemiology of antimicrobial resistance in Salmonella isolated from pork, chicken meat and humans in Thailand. *Southeast Asian J Trop.Med Public Health, 36*, 1510-1515.

Angulo, F. J., Nargund, V. N., & Chiller, T. C. (2004). Evidence of an Association Between Use of Anti-microbial Agents in Food Animals and Anti-microbial Resistance Among Bacteria Isolated from Humans and the Human Health Consequences of Such Resistance. *Journal of Veterinary Medicine Series B, 51,* 374-379.

Animal Health Institute. (2000). Survey indicates most antibiotics used in animals are used for treating and preventing disease (press release February 14, 2000) Retrieved September 15, 2008, from http:// www.ahi.org

AREI. (2006). Chapter 4.9: U.S. Organic Agriculture. Retrieved October 3, 2008, from http://www.ers.usda.gov/publications/arei/eib16/Chapter4/4.9/

Ault, K. A., Kennedy, M., Seoud, M. A., & Reiss, R. (1993). Maternal and Neonatal Infection With Salmonella heidelberg : A Case Report. *Infect.Dis.Obstet.Gynecol., 1,* 46-48.

Bager, F., Madsen, M., Christensen, J., & Aarestrup, F. M. (1997). Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish poultry and pig farms. *Preventive Veterinary Medicine, 31,* 95-112.

Baggesen, D. L., Sandvang, D., & Aarestrup, F. M. (2000). Characterization of Salmonella enterica Serovar Typhimurium DT104 Isolated from Denmark and Comparison with Isolates from Europe and the United States. *Journal of Clinical Microbiology, 38,* 1581-1586.

Baloda, S. B., Christensen, L., & Trajcevska, S. (2001). Persistence of a Salmonella enterica Serovar Typhimurium DT12 Clone in a Piggery and in Agricultural Soil Amended with Salmonella-Contaminated Slurry. *Applied and Environmental Microbiology, 67,* 2859-2862.

Baquero, F., Negri, M., Morosini, M., & Blaízquez, J. S. (1998). Antibiotic ÇÉ Selective Environments. *Clinical Infectious Diseases, 27,* S5-S11.

Barza, M. & Travers, K. (2002). Excess Infections Due to Antimicrobial Resistance: The "Attributable Fraction". *Clinical Infectious Diseases, 34,* S126.

Baumler, A. J., Tsolis, R. M., Ficht, T. A., & Adams, L. G. (1998). Evolution of Host Adaptation in Salmonella enterica. *Infection and Immunity, 66,* 4579-4587.

Berchieri, A., Jr., Turco, W. C., Paiva, J. B., Oliveira, G. H., & Sterzo, E. V. (2006). Evaluation of isopathic treatment of Salmonella enteritidis in poultry. *Homeopathy., 95,* 94-97.

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection, 4,* 413-423.

Blanch, A. R., Caplin, J. L., Iversen, A., Kuhn, I., Manero, A., Taylor, H. D. et al. (2003). Comparison of enterococcal populations related to urban and hospital wastewater in various climatic and geographic European regions. *Journal of Applied Microbiology, 94,* 994-1002.

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). Salmonella Nomenclature. *Journal of Clinical Microbiology, 38,* 2465-2467.

Bywater, R. J. (2004). Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. *J Vet.Med B Infect.Dis.Vet.Public Health, 51,* 361-363.

Carr, L. E., Mallinson, E. T., Tate, C. R., Miller, R. G., Russek-Cohen, E., Stewart, L. E. et al. Prevalence of Salmonella in Broiler Flocks: Effect of Litter Water Activity, House Construction, and Watering Devices. *Avian Diseases, 39,* 39-44.

Centers for Disease Control. (2005). Typhoid Fever. Retrieved March 13, 2008, from http://www.cdc.gov/ncidod/dbmd/diseaseinfo/TyphoidFever_g.htm

Centers for Disease Control. (2008a). Get Smart: Know When Antibiotics Work: Glossary. Retrieved October 7, 2008, from http://www.cdc.gov/drugresistance/community/glossary.htm

Centers for Disease Control. (2008b). Information for the public about VRE. Retrieved October 3, 2008, from http://www.cdc.gov/ncidod/dhqp/ar_vre_publicfaq.html

Chapin, A., Rule, A., Gibson, K., Buckley, T., & Schwab, K. (2005). Airborne Multidrug-Resistant Bacteria Isolated from a Concentrated Swine Feeding Operation. *Environmental Health Perspectives, 113,* 137-142.

Commission of the European Communities. 2003. Commission Regulation (EC) No 2277/2003 of December 22, *Official Journal of the European Union*: L 336/71

Coburn, B., Grassl, G. A., & Finlay, B. B. (2006). Salmonella, the host and disease: a brief review. *Immunol Cell Biol, 85,* 112-118.

Crum, N. F. (2003). Current trends in typhoid Fever. *Curr.Gastroenterol.Rep., 5,* 279-286.

Cui, S., Ge, B., Zheng, J., & Meng, J. (2005). Prevalence and Antimicrobial Resistance of Campylobacter spp. and Salmonella Serovars in Organic Chickens from Maryland Retail Stores. *Applied and Environmental Microbiology, 71,* 4108-4111.

Cunha, B. A. (2004). Osler on typhoid fever: differentiating typhoid from typhus and malaria. *Infectious Disease Clinics of North America, 18,* 111-125.

D'Souza, R. M., Becker, N. G., Hall, G., & Moodie, K. B. (2004). Does ambient temperature affect foodborne disease? *Epidemiology, 15,* 86-92.

Davies, R. H. & Wray, C. (1999). Studies of Contamination of Three Broiler Breeder Houses with Salmonella enteritidis before and after Cleansing and Disinfection. *Avian Diseases, 40,* 626-633.

Demerrit, L. (2004). "Organic Pathways," *[N]sight* 6(2). Belleview, WA. Hartman Group, Inc.

Dimitri, C. & Greene, C. (2002). *Recent Growth Patterns in the U.S. Organic Foods Market.* Washington D.C.: United States Department of Agriculture and Economic Research Service Bulletin #777.

Donabedian, S. M., Thal, L. A., Hershberger, E., Perri, M. B., Chow, J. W., Bartlett, P. et al. (2003). Molecular Characterization of Gentamicin-Resistant

Enterococci in the United States: Evidence of Spread from Animals to Humans through Food. *Journal of Clinical Microbiology, 41,* 1109-1113.

Edmond, M. B., Ober, J. F., Dawson, J. D., Weinbaum, D. L., & Wenzel, R. P. (1996). Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. *Clin.Infect.Dis., 23,* 1234-1239.

Edwards, R. A., Olsen, G. J., & Maloy, S. R. (2002). Comparative genomics of closely related salmonellae. *Trends in Microbiology, 10,* 94-99.

Eliopoulos, G. M., Wennersten, C., Zighelboim-Daum, S., Reiszner, E., Goldmann, D., & Moellering, R. C. (1988). High-level resistance to gentamicin in clinical isolates of Streptococcus (Enterococcus) faecium. *Antimicrob.Agents Chemother., 32,* 1528-1532.

Ellermeier CD & Slauch J.M. (2006). Genus Salmonella. In: Dworkin, M.D.(ed.). 2006. The Prokaryotes: A Handbook on the Biology of Bacteria. SpringerPress. New York

Facklam, R. (2002). History,Taxonomy, Biochemical Characteristics, and Antibiotic Susceptibility Testing of Enterococci. In: Gilmore, M. S. (ed.). 2002. The enterococci: pathogenesis, molecular biology, and antibiotic resistance. ASM Press, Washington, DC

Food and Drug Administration. (1999). APPROVAL OF SYNERCID FOR CERTAIN VANCOMYCIN RESISTANT INFECTIONS (T99-44). Washington, DC: Author. Retrieved from http://www.fda.gov/bbs/topics/ANSWERS/ANS00976.html

Food and Drug Administration. (2004). FDA Approved Animal Drug ProductsDrug Information Laboratory, Virginia/Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Garcia-Migura, L., Pleydell, E., Barnes, S., Davies, R. H., & Liebana, E. (2005). Characterization of Vancomycin-Resistant Enterococcus faecium Isolates from Broiler Poultry and Pig Farms in England and Wales. *Journal of Clinical Microbiology, 43*, 3283-3289.

Gilchrist, M. J., Greko, C., Wallinga, D. B., Beran, G. W., Riley, D. G., & Thorne, P. S. (2007). The Potential Role of Concentrated Animal Feeding Operations in Infectious Disease Epidemics and Antibiotic Resistance. *Environmental Health Perspectives, 115*, 313-316.

Giraffa, G. (2002). Enterococci from foods. *FEMS Microbiology Reviews, 26*, 163-171.

GovTrack. (2009). *Preservation of Antibiotics for Medical Treatment Act of 2009*. H.R. 1549/S. 619. Washington, DC: Author. Retrieved from http://www.govtrack.us/congress/bill.xpd?bill=h111-1549

Gorbach, S. L. (2001). Antimicrobial use in animal feed--time to stop. N.Engl.J.Med. 345:1202-1203.

Graham, J. P., Boland, J. J., & Silbergeld, E. (2007). Growth Promoting Antibiotics in Food Animal Production: An Economic Analysis. *Public Health Rep., 122*, 79-87.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S. et al. (2003). Emergence of Multidrug-Resistant Salmonella enterica Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States. *Journal of Infectious Diseases, 188,* 1707-1716.

Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R. V., Rossiter, S. P., Friedman, C. R. et al. (2004). Antimicrobial Resistance among Campylobacter Strains, United States, 1997-2001. Emerging Infectious Diseases 10[6], 1102-1109. Centers for Disease Control & Prevention (CDC).
Ref Type: Generic

Hardie JM. (1986) Genus Streptococcus Rosenbach (1884). In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, Vol 2. Wdhams and Wilkens, Baltimore, p 1043-1071

Harrison, P. and Lederberg, J. (eds). 1998. "Antimicrobial Resistance: Issues and Options." Workshop Report, Forum on Emerging Infections, Division of Health and Sciences Policy, Institute of Medicine. National Academy Press: Washington, D.C

Hayes, J. R., McIntosh, A. C., Qaiyumi, S., Johnson, J. A., English, L. L., Carr, L. E. et al. (2001). High-Frequency Recovery of Quinupristin-Dalfopristin-Resistant Enterococcus faecium Isolates from the Poultry Production Environment. *Journal of Clinical Microbiology, 39,* 2298-2299.

Hayes, J. R., English, L. L., Carr, L. E., Wagner, D. D., & Joseph, S. W. (2004). Multiple-Antibiotic Resistance of Enterococcus spp. Isolated from Commercial

Poultry Production Environments. *Applied and Environmental Microbiology, 70,* 6005-6011.

Hayes, J. R., English, L. L., Carter, P. J., Proescholdt, T., Lee, K. Y., Wagner, D. D. et al. (2003). Prevalence and Antimicrobial Resistance of Enterococcus Species Isolated from Retail Meats. *Applied and Environmental Microbiology, 69,* 7153-7160.

Heuer, O. E., K. Pedersen, J. S. Andersen, and M. Madsen. 2001. Prevalence and antimicrobial susceptibility of thermophilic Campylobacter in organic and conventional broiler flocks. Lett.Appl.Microbiol. 33:269-274.

Heuer, O. E., A. M. Hammerum, P. Collignon, and H. C. Wegener. 2006. Human health hazard from antimicrobial-resistant enterococci in animals and food. Clin.Infect.Dis. 43:911-916.

Hitti, W. & Wolff, M. (2005). Two cases of multidrug-resistant Nocardia farcinica infection in immunosuppressed patients and implications for empiric therapy. *European Journal of Clinical Microbiology & Infectious Diseases, 24,* 142-144.

Humphrey, T. (2001). The Spread and Persistence of Campylobacter and Salmonella in the Domestic Kitchen. *Journal of Infection, 43,* 50-53.

Huycke, M. M., Sahm, D. F., & Gilmore, M. S. (1998). Multiple-drug resistant enterococci: The nature of the problem and an agenda for the future. *Emerging Infectious Diseases, 4,* 239.

Idris, U., J. Lu, M. Maier, S. Sanchez, C. L. Hofacre, B. G. Harmon, J. J. Maurer, and M. D. Lee. (2006). Dissemination of fluoroquinolone-resistant Campylobacter spp. within an integrated commercial poultry production system. Appl.Environ.Microbiol. 72:3441-3447.

Ishiguro, F., Fuseda, T., Omoya, S., Kyota, Y., Mochizuki, M., Izumiya, H. et al. (2004). [First report of multidrug-resistant Salmonella Newport in Japan]. *Kansenshogaku Zasshi, 78,* 989-990.

Institute of Medicine. (1998). Antimicrobial Resistance: Issues and Options, Workshop Report, Forum on Emerging InfectionsNational Academy Press, Washington, D.C.

Jacob, J. P., Griggs, J. P., & Bender, J. B. (2008). Characterization of Small-Scale Antibiotic-Free Broiler Production in Minnesota. *The Journal of Applied Poultry Research, 17,* 412-420.

Jett, B. D., Huycke, M. M., & Gilmore, M. S. (1994). Virulence of enterococci. *Clinical Microbiology Reviews, 7,* 462-478.

Johnson, A. P. (1994). The pathogenicity of enterococci. *Journal of Antimicrobial Chemotherapy, 33,* 1083-1089.

Jones, P. W. & Twigg, G. I. (1976). Salmonellosis in Wild Mammals. *The Journal of Hygiene, 77,* 51-54.

Joseph, S. W., Wagner, D. D., Hayes, J. R., Sapkota, A. R., Cullen, P., Paramadhas, M., Hulett, M., Hooberman, B., and Carr, L. E. Comparison of the prevalence of multiple antibiotic resistance of Salmonella spp and Enterococcus faecium isolated from organic, antibiotic-free and conventional U.S. poultry farms.
Ref Type: Generic

Jukes, T. H., Hill D.C & Branion, D.H. (1956). Effect of feeding antibiotics on the intestinal tract of the chick. Poult. Sci.35:716–723.

Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *CMAJ., 159,* 1129-1136.

Kühn, I., Iversen, A., Burman, L. G., Olsson-Liljequist, B., Franklin, A., Finn, M. et al. (2000). Epidemiology and ecology of enterococci, with special reference to antibiotic resistant strains, in animals, humans and the environment: Example of an ongoing project within the European research programme. *International Journal of Antimicrobial Agents, 14,* 337-342.

Kummerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy, 54,* 311-320.

Levinson, W. (2006). Review of Medical Microbiology and Immunology. San Francisco,California: Lange Medical Books/ McGraw-Hill Medical Publishing Division

Levy, S. B., FitzGerald, G. B., & Macone, A. B. (1976). Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *The New England Journal of Medicine, 295,* 583-588.

Levy, S. B. (1998). The challenge of antibiotic resistance. *Scientific American, 278,* 46.

Levy, S. B. & Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med*.

Logue, C. M., Sherwood, J. S., Olah, P. A., Elijah, L. M., & Dockter, M. R. (2003). The incidence of antimicrobial-resistant Salmonella spp. on freshly processed poultry from US Midwestern processing plants*. *Journal of Applied Microbiology, 94,* 16-24.

Luangtongkum, T., Morishita, T. Y., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of Conventional and Organic Production Practices on the Prevalence and Antimicrobial Resistance of Campylobacter spp. in Poultry. *Applied and Environmental Microbiology, 72,* 3600-3607.

MacCallum, W. G. and T. W. Hastings. 1899. A case of acute endocarditis caused by Micrococcus zymogenes (nov. spec.), with a description of the microorganism. J.Exp.Medicine 4:521-534.

Madhi, S., Petersen, K., Madhi, A., Khoosal, M., & Klugman, K. (2000). Increased Disease Burden and Antibiotic Resistance of Bacteria Causing Severe Community□ÇÉAcquired Lower Respiratory Tract Infections in Human Immunodeficiency Virus Type 1□ÇôInfected Children. *Clinical Infectious Diseases, 31,* 170-176.

Manson, J. M., Keis, S., Smith, J. M. B., & Cook, G. M. (2003). A Clonal Lineage of VanA-Type Enterococcus faecalis Predominates in Vancomycin-Resistant Enterococci Isolated in New Zealand. *Antimicrob.Agents Chemother., 47,* 204-210.

Maskalyk, J. (2003). Typhoid fever. *CMAJ., 169,* 132.

Mathew, A. G., Cissell, R., & Liamthong, S. (2007). Antibiotic Resistance in Bacteria Associated with Food Animals: A United States Perspective of Livestock Production. *Foodborne Pathogens and Disease, 4,* 115-133.

Mazel, D. & Davies, J. (1999). Antibiotic resistance in microbes. *Cellular and Molecular Life Sciences (CMLS), 56,* 742-754.

McGowan, J. E., Jr. (1983). Antimicrobial Resistance in Hospital Organisms and Its Relation to Antibiotic Use. *Reviews of Infectious Diseases, 5,* 1033-1048.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C. et al. (2000). Food-Related Illness and Death in the United States. *Journal of Environmental Health, 62,* 9.

Mellon M, Benbrook C, Benbrook KL. *Hogging It! Estimates of Antimicrobial Abuse in Livestock.* Washington, DC: Union of Concerned Scientists; 2001

Mian, L. S., Maag, H., & Tacal, J. V. (2002). Isolation of Salmonella from muscoid flies at commercial animal establishments in San Bernardino County, California. *J Vector.Ecol., 27,* 82-85.

Miranda, J. M., Guarddon, M., Mondragon, A., Vazquez, B. I., Fente, C. A., Cepeda, A. et al. (2007). Antimicrobial resistance in Enterococcus spp. strains isolated from organic chicken, conventional chicken, and turkey meat: a comparative survey. *J Food Prot., 70,* 1021-1024.

Molbak, K. 2005. Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. Clin.Infect.Dis. 41:1613-1620.

Morgan, K. & Murdoch, J. (2000). Organic vs. conventional agriculture: knowledge, power and innovation in the food chain. *Geoforum, 31,* 159-173.

Morris, J. G., Shay, D. K., Hebden, J. N., McCarter, R. J., Perdue, B. E., Jarvis, W. et al. (1995). Enterococci Resistant to Multiple Antimicrobial Agents, Including Vancomycin: Establishment of Endemicity in a University Medical Center. *Annals of Internal Medicine, 123,* 250-259.

Motarjemi, Y. and Adams, M. 2006. Introduction to emerging foodborne pathogens. pp. xviii --- xxii. Woodhead Publishing Ltd. Cambridge, England.

Mundt, J. O. (1961). Occurrence of Enterococci: Bud, Blossom, and Soil Studies. *Appl.Microbiol., 9,* 541-544.

Mundy, L. M., Sahm, D. F., & Gilmore, M. (2000). Relationships between Enterococcal Virulence and Antimicrobial Resistance. *Clinical Microbiology Reviews, 13,* 513-522.

Murray, B. E. (1990). The life and times of the Enterococcus. *Clinical Microbiology Reviews, 3,* 46-65.

Murray, B. E. (1998). Diversity among multidrug-resistant Enterococci. *Emerging Infectious Diseases, 4,* 37.

Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, Stratton C. (2008), Manual of clinical microbiology, 9th edition:manual of clinical microbiology, 9th edition. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. Jan;46(1):153-4.

Nadelman, R. B., Nowakowski, J., Fish, D., Falco, R. C., Freeman, K., McKenna, D. et al. (2001). Prophylaxis with Single-Dose Doxycycline for the Prevention of Lyme Disease after an Ixodes scapularis Tick Bite. *The New England Journal of Medicine, 345,* 79-84.

National Agricultural Library. (2007). Organic Production/ Organic Food: Information Access Tools. Retrieved March 20, 2009, from http://www.nal.usda.gov/afsic/pubs/ofp/ofp.shtml

National Agricultural Statistics Service. (2006). Organic Agricultural Production in 2005. Retrieved September 16, 2008, from www.ers.usda.gov/data/organic

National Agricultural Statistics Service. (2008). Poultry-Production and Value 2007:Summary. Retrieved September 16, 2008, from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1367

National Antimicrobial Resistance Monitoring System. (2005). Enteric Bacteria. Retrieved November, 22, 2008, from http://www.cdc.gov/NARMS/annual/2005/NARMSAnnualReport2005.pdf

National Antimicrobial Resistance Monitoring System. (2009). National Antimicrobial Resistance Monitoring System (NARMS). Retrieved June, 22, 2008, from http://www.fda.gov/cvm/narms_pg.html

National Research Council. 1999. The Use of Drugs in Food Animals: Benefits and RisksNational Academy Press, Washington, D.C.

Natural Food Merchandiser. (2006). Organic Food: Organic Meat. Retrieved September 3, 2008, from http://www.nfm-online.com/

Nicolle, L. E., Strausbaugh, L. J., & Garibaldi, R. A. (1996). Infections and antibiotic resistance in nursing homes. *Clinical Microbiology Reviews, 9,* 1-17.

Noble, W. C., Virani, Z., & Cree, R. G. (1992). Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. *FEMS Microbiol.Lett., 72,* 195-198.

Normark, B. H. & Normark, S. (2002). Evolution and spread of antibiotic resistance. *Journal of Internal Medicine, 252,* 91-106.

Nutrition Business Journal. (NBJ). (2004). *NBJ's Organic Foods Report 2004.* Penton Media, Inc

North Carolina Cooperative Extension Service. (2007). The North Carolina Poultry Industry. Retreived October 2, 2008, from http://www.ces.ncsu.edu/depts/poulsci/tech_manuals/poultry_industry.html

Oberholtzer, L., C. Dimitri, and C. Greene. (2005). Price premiums hold on as U.S. organic produce market expands. Economic Research Service, U.S. Department of Agriculture, Washington, D.C.

Ohio Department of Health. (2007). Vancomycin Resistant Enterococcus. Retrieved October, 7, 2008, from http://www.odh.ohio.gov/pdf/IDCM/vre.pdf

Ohl, M. E. & Miller, S. I. (2001). SALMONELLA: A Model for Bacterial Pathogenesis. *Annual Review of Medicine*, *52*, 259.

Organic Trade Association. (2006).U.S. Organic Industry Overview. Retrieved October 3, 2008, from http://www.ota.com/pics/documents/short%20overview%20MMS.pdf

Orla-Jensen, S. 1919. The lactic acid bacteria. Mem. Acad. R.Soc. Danemark Sect. Sci. Ser. 8 5:81-197.

Overbeke, I. V., Duchateau, L., Zutter, L. D., Albers, G., & Ducatelle, R. (2006). A Comparison Survey of Organic and Conventional Broiler Chickens for Infectious Agents Affecting Health and Food Safety. *Avian Diseases, 50*, 196-200.

Piekus, J., Franciosini, M.P., Proietti, P.C., Reich, E. et al. (2008). Preliminary Investigations on *Salmonella* spp. Incidence in Meat Chicken Farms in Italy, Germany, Lithuania and the Netherlands. International Journal of Poultry Science 7 (8): 813-817

Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., & Frey, J. (2005). Microarray-Based Detection of 90 Antibiotic Resistance Genes of Gram-Positive Bacteria. *Journal of Clinical Microbiology, 43,* 2291-2302.

Porwollik, S., Wong, R. M.-Y., & McClelland, M. (2002). Evolutionary genomics of Salmonella: Gene acquisitions revealed by microarray analysis. *Proceedings of the National Academy of Sciences of the United States of America, 99,* 8956-8961.

Prescott, J. F. (1999). A Review of Antimicrobial Resistance in the Food Chain. *Can.Vet.J, 40,* 199.

Price, L. B., Lackey, L. G., Vailes, R., & Silbergeld, E. (2007). The persistence of fluoroquinolone-resistant Campylobacter in poultry production. *Environ.Health Perspect., 115,* 1035-1039.

Price, L. B., Johnson, E., Vailes, R., & Silbergeld, E. (2005). Fluoroquinolone-Resistant CampylobacterIsolates from Conventional and Antibiotic-Free Chicken Products. *Environmental Health Perspectives, 113,* 557-560.

Ramsey, A. & Zilberberg, M. (2009). Secular Trends of Hospitalization With Vancomycin□ÇÉResistant Enterococcus Infection in the United States, 2000□ÇÉ2006-á□Çó□Çé. *Infection Control and Hospital Epidemiology, 30,* 184-186.

Ray, K. A., Warnick, L. D., Mitchell, R. M., Kaneene, J. B., Ruegg, P. L., Wells, S. J. et al. (2006). Antimicrobial Susceptibility of Salmonella from Organic and Conventional Dairy Farms. *Journal of Dairy Science, 89,* 2038-2050.

Rice, E. W., Messer, J. W., Johnson, C. H., & Reasoner, D. J. (1995). Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. *Applied and Environmental Microbiology, 61,* 374-376.

Richards, M. J., Edwards, J. R., Culver, D. H., & Gaynes, R. P. (2000). Nosocomial Infections in Combined Medical□ÇÉSurgical Intensive Care Units in the United States-á□Çó□Çé. *Infection Control and Hospital Epidemiology, 21,* 510-515.

Ruiz, J., Nunez, M. L., Diaz, J., Sempere, M. A., Gomez, J., & Usera, M. A. (1996). Note: comparison of media for the isolation of lactose-positive Salmonella. *J Appl.Bacteriol., 81,* 571-574.

Schaberg, D. R., Culver, D. H., & Gaynes, R. P. (1991). Major trends in the microbial etiology of nosocomial infection. *Am.J Med, 91,* 72S-75S.

Schleifer, K. H. 1984. Gram-positive cocci, p. 999-1103. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.

Schleifer, K. H., and R. Kilpper-Balz. 1984. Transfer of Streptococcus faecalis and Streptococcus faecium to the genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. Int. J. Syst. Bacteriol. 34:31-34.

Seepersadsingh, N. & Adesiyun, A. A. (2003). Prevalence and Antimicrobial Resistance of Salmonella spp. in Pet Mammals, Reptiles, Fish Aquarium Water, and Birds in Trinidad. *Journal of Veterinary Medicine Series B, 50,* 488-493.

Shea, K. M. (2003). Antibiotic Resistance: What Is the Impact of Agricultural Uses of Antibiotics on Children's Health? *Pediatrics, 112,* 253-258.

Shere K.D., Goldberg M.B. & Rubin R.H. (1998). Salmonella infections. In: *Infectious Diseases* (eds S.L. Gorbach, J.G. Bartlett & N.R. Blacklow), pp. 699–712. W.B. Saunders, Philadelphia.

Sherman, J. M. (1937). THE STREPTOCOCCI. *Microbiology and Molecular Biology Reviews, 1,* 3-97.

Silbergeld, E. K., Graham, J., & Price, L. B. (2008). Industrial Food Animal Production, Antimicrobial Resistance, and Human Health. *Annual Review of Public Health, 29,* 151-169.

Singer, R. S. & Hofacre, C. L. (2006). Potential Impacts of Antibiotic Use in Poultry Production. *Avian Diseases, 50,* 161-172.

Slauch J, Taylor R, Maloy S. (1997). Survival in a cruel world: how Vibrio cholerae and *Salmonella* respond to an unwilling host. *Genes and Development*, 11:1761-1774.

Smith, D. L., Dushoff, J., & Morris, J. G. (2005). Agricultural Antibiotics and Human Health. *PLoS.Med, 2*.

Smith, D. L., Dushoff, J., & Morris, J. G. (2005). Agricultural Antibiotics and Human Health. *PLoS.Med, 2*.

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., & Morris, J. G. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proceedings of the National Academy of Sciences of the United States of America, 99,* 6434-6439.

Spellberg, B., Guidos, R., Gilbert, D., Bradley, J., Boucher, H. W., Scheld, W. M. et al. (2008). The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin.Infect.Dis., 46,* 155-164.

Strausbaugh, L. J., Crossley, K. B., Nurse, B. A., & Thrupp, L. D. (1996). Antimicrobial resistance in long-term-care facilities. *Infection Control and Hospital Epidemiology, 17,* 129-140.

Tannock, G.W. & Cook, G., 2002. Enterococci as Members of the Intestinal Microflora of Humans. In: Gilmore, M. S. (ed.). 2002. The enterococci: pathogenesis, molecular biology, and antibiotic resistance. ASM Press, Washington, DC

Thiercelin, E. 1899. Sur un diplocoque saprophyte de l'intestin susceptible de devenir pathogene. C.R.Soc.Biol. 5:269-271.

Threlfall, E. J. (2000). Epidemic Salmonella typhimurium DT 104--a truly international multiresistant clone. *Journal of Antimicrobial Chemotherapy, 46,* 7-10.

Threlfall, E. J., Ward, L. R., Frost, J. A., & Willshaw, G. A. (2000). The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology, 62,* 1-5.

Threlfall, E. J. (2002). Antimicrobial drug resistance in Salmonella: problems and perspectives in food- and water-borne infections. *FEMS Microbiology Reviews, 26,* 141-148.

United States Department of Agriculture: Economic Research Service. (1999). Poultry Production in the United States. Retrieved September 16, 2008, from http://www.ers.usda.gov/publications/aib748/aib748b.pdf

United States Department of Agriculture: Economic Research Service. (2002). Organic Production. Retrieved September 14, 2008, from *www.ers.usda.gov/Data/organic*

United States Department of Agriculture: Economic Research Service. (2006). U.S. Broiler Industry: Background Statistics and Information. Retrieved September, 16, 2008 from http://www.ers.usda.gov/News/broilercoverage.htm

United States Department of Agriculture: Economic Research Service. (2007). Poultry Production in the United States. Retrieved September 16, 2008, from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1367

United States Department of Agriculture: Economic Research Service. (2008). Briefing Room: Poultry and Eggs. Retrieved September, 16, 2008, from http://www.ers.usda.gov/briefing/poultry/

United States Department of Agriculture: Economic Research Service. (2008b). Dietary Assessment of Major Trends in U.S. Food Consumption, 1970-2005. Retrieved September 20, 2008, from http://www.ers.usda.gov/Publications/EIB33/EIB33.pdf

United States Department of Agriculture: National Organic Program. (2003). Organic Program Overview. Retrieved September 14, 2008, from http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateA&navID=NationalOrganicProgram&leftNav=NationalOrganicProgram&page=NOPNationalOrganicProgramHome&acct=nop

U.S. Department of Health and Human Services. (2005). *Medicaid drug price comparisons: Average manufacturer price to published prices* (OIG publication No. OEI-05-05- 00240). Washington, DC: Author. Retrieved from http://www.oig.hhs.gov/oei/reports/oei-05-05-00240.pdf

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, G., Bernard, S. et al. (2001). Host Adapted Serotypes of Salmonella enterica. *Epidemiology and Infection, 125,* 229-255.

van den Bogaard, A. E. & Stobberingh, E. E. (1999). Antibiotic Usage in Animals: Impact on Bacterial Resistance and Public Health. *Drugs, 58,* 589-607.

van den Bogaard, A. E. & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. *International Journal of Antimicrobial Agents, 14,* 327-335.

Visek, W. J. (1978). The Mode of Growth Promotion by Antibiotics. *Journal of Animal Science, 46,* 1447-1469.

Voetsch, A. C., Van Gilder, T. J., Angulo, F. J., Farley, M. M., Shallow, S., Marcus, R. et al. (2004). FoodNet Estimate of the Burden of Illness Caused by Nontyphoidal Salmonella Infections in the United States. *Clinical Infectious Diseases, 38,* S127-S134.

Wegener, H. C., Aarestrup, F. M., Jensen, L. B., Hammerum, A. M., & Bager, F. (1999). Use of Antimicrobial Growth Promoters in Food Animals and Enterococcus faecium Resistance to Therapeutic Antimicrobial Drugs in Europe. *Emerging Infectious Diseases, 5,* 329-335.

Wegener, H. C. (2003). Antibiotics in animal feed and their role in resistance development. *Current Opinion in Microbiology, 6,* 439-445.

Weinstein, R. A. (1998). Nosocomial infection update. *Emerging Infectious Diseases, 4,* 416.

White, D. G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S. et al. (2001). The Isolation of Antibiotic-Resistant Salmonella from Retail Ground Meats. *The New England Journal of Medicine, 345,* 1147-1154.

Windhourst, H. W. (2006). Changes in poultry production and trade worldwide. *World's Poultry Science Journal, 62,* 585-602.

Winfield, M. D. & Groisman, E. A. (2003). Role of Nonhost Environments in the Lifestyles of Salmonella and Escherichia coli. *Applied and Environmental Microbiology, 69,* 3687-3694.

Witte, W. (2001). Selective pressure by antibiotic use in livestock. *International Journal of Antimicrobial Agents, 16,* 19-24.

World Health Organization. (2002). Antimicrobial resistance (Fact Sheet N°194). World Health Media Centre. Retrieved from

http://www.who.int/mediacentre/factsheets/fs194/en/

Yu, H. & Bruno, J. G. (1996). Immunomagnetic-electrochemiluminescent detection of Escherichia coli O157 and Salmonella typhimurium in foods and environmental water samples. *Applied and Environmental Microbiology, 62,* 587-592.

ERINNA L. KINNEY

University of Maryland College Park
SPH Bldg 255
Environmental Health Laboratories
College Park, MD 20742
Phone (301) 403-2575 (work)
Phone (410) 448-2476
Email: erinna.kinney@gmail.com

OBJECTIVE

To pursue career and educational opportunities in environmental and food microbiology, leading to an enhanced intellectual knowledge and experiential research experience in Environmental Health Science

EDUCATION

Aug 2007-2009, University of Maryland College Park-School of Public Health, Candidate for M.P.H. Environmental Health Sciences, expected 03/2009
Summa Cum Laude, GPA 3.98 on 4.0 scale

Aug 1997-2001, Clark Atlanta University, Atlanta, Georgia, B.S. Biology, Magna Cum Laude, GPA 3.77 on 4.0 scale

RELEVANT COURSEWORK

Pathogenic Microbiology, Environmental Health Microbiology, Fundamentals of Epidemiology, Principles of Toxicology, Environmental and Occupational Diseases, Wildlife Diseases, and Biostatistics

RESEARCH AND WORK EXPERIENCE

FDA Center for Veterinary Medicine, **Dates Employed: 06/2008-08/2008**
Division of Animal and Food Microbiology
1600 Muirkirk Road **Laurel, MD 30333**

FDA CVM Intern
Major Advisor- Dr. Patrick McDermott

- Performed applied microbial research within Division of Animal and Food Microbiology (DAFM) at the Office of Research on elucidating effects of antimicrobial resistance in pathogenic and commensal bacterial organisms derived from conventional and organic poultry environments
- Completed objectives:
  - Biochemically screened *Enterococcus spp*. (n=313) isolates and *Salmonella spp.* (n=131) isolates from the 2008 UMD/Penn State Poultry Farm Study

- Identified *Enterococcus spp*. (n=313) isolates and *Salmonella spp.* (n=131) isolated from the 2008 UMD/Penn State Poultry Farm Study via Vitek ®system
- Performed antimicrobial susceptibility testing on *Enterococcus spp.* (n=265) isolates and *Salmonella spp.* (n=120) isolates from the 2008 UMD/Penn State Poultry Farm Study using the Sensititre ™system
- Serotyped *Salmonella spp.* (n=121) isolates from the 2008 UMD/Penn State Poultry Farm Study

University of Maryland College Park, School of Public Health
Maryland Institute of Applied Environmental Health **Dates Employed: 07/2007-05/2009**
College Park, MD 20742

Graduate Research Assistant
Major Advisor- Dr. Amy R. Sapkota

- Conducted environmental microbiology laboratory research that encompasses the isolation, cultivation, and microbial analysis of environmental samples
  - Research Thesis:"ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SUCSEPTIBILITY ANALYSIS OF ENTEROCOCCCUS SPP. AND SALMONELLA SPP. FROM CONVENTIONA POULTRY FARM ENVIRONMENTS TRANSITIONING TO ORGANIC POULTRY PRODUCTION
- Characterized microbial loads of *Salmonella* spp. and *Enterococcus* spp. recovered from poultry farms converting from conventional to organic practices and discontinue the use of antibiotics
- Quantified on-farm antibiotic resistance patterns of *Salmonella* spp. and *Enterococcus* spp. during conversion process
- Analyzed the significance of environmental variables on the prevalence of on-farm microbial load levels and antibiotic resistance patterns of *Salmonella* spp., and *Enterococcus* spp. derived from the conversion of conventional to organic poultry production practices
- Developed field sampling protocols and laboratory standard operating procedures for UMD Poultry Farm Study, UMD/JHU Yakima Valley Dust Study, and UMD Spray Irrigation Study in the detection of microbial organisms in the environment
- Responsible for maintenance of the laboratory equipment and facility

Centers for Disease Control
National Center for Zoonotic, Vector-borne, and Enteric Disease
1600 Clifton Road **Dates Employed: 02/2007-09/2007**
Atlanta, GA 30333

Laboratory Research Intern
Enteric Disease Reference Laboratory
Major Advisor- Dr. Cheryl Tarr

- Aid in the development of a Multiplex Assay and rpoB sequence determination for identification Campylobacter isolates
- Utilize genomic and molecular tools for diagnostic application in the identification of Campylobacter
- Tangible Outcomes

- The two-step approach will allow rapid and accurate discrimination of the *Campy* species that are implicated in human infections.
- Multiple species can be discriminated with a single PCR assay.
- The assay will be designed as a rapid classification tool for clinical laboratories, but the markers may be adapted for use virulence genes as markers for pathogenic species

University of Georgia **Dates Employed: 09/2003-2006**
1570 Athens Street **Athens, GA, 30605**

Graduate Research Assistant
Major Advisor: Dr. Amy Rosemond

- Perform duties as a research assistant in the Rosemond Lab
- Research project: Effects of Nutrient Enrichment on Decomposition Rates and Invertebrate Assemblages in Headwater Streams, (Coweeta National Hydrological Laboratory, NC)

Atlanta Outward Bound Center AmeriCorps **Dates Employed: 09/2002-06/2003**
3790 Market Street **Clarkston, GA 30021**

EcoWatch AmeriCorps Member

- A ten-month commitment through United States Americorps Program and the Atlanta Upward Bound Center to complete 1700 hours of environmentally oriented community service in Georgia
- Performed biological and chemical water testing under Georgia's Adopt-A-Stream Program
- Constructed and maintenance of nature trails, organic community gardens, and conservation projects
- Instructed environmental education classes and programs for K-12 students in Georgia
- Developed and operated an environmentally focused After-School Program at Clairemont Elementary School

United States Environmental Protection Agency **Dates Employed: 08/2001-07/2002**
1200 Pennsylvania Ave, NW **Washington D.C., 20460**

Clean Air Program Analyst

- Conducted studies and analyses in the formulation of the Clean Air Budget in the Office of Program Management Operations for the Office of Air and Radiation
- Reviewed project and program effectiveness in achieving Goal 6: Reduction of Global and Cross Border Environmental Risks through preparation of the U.S. EPA 2001 Annual Report for OAR

AWARDS, HONORS AND PROFESSIONAL MEMBERSHIPS

- Dean's Scholar : UMD School of Public Health (2009)
- Golden Key National Honor Society (2008- Present)
- University of Maryland College Park Dean's List (2007-Present)
- Clark Atlanta University Dean's List (4 years)
- American Public Health Association Student Member (2007- Present)
- American Society of Microbiology Student Member (2007-Present)
- Association for the Advancement of Science (AAS) Student Member (2005-Present)
- Ecological Society of America Professional Member (2001-2002) Student Member (2005-Present)
- Sierra Club Member (2003-Present)
- CSX Corporation/ National Audubon Society Scholar (1999-2002)
- ACWA Outstanding Scholar Program EPA (2001-2002)