Antimicrobial Resistance and Zoonotic Outbreaks of *Salmonella enterica* in the United States, 2015–2018

Erin Frey, DVM

A Master's Paper submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Public Health in the Public Health Leadership Program

Chapel Hill
Fall 2018

Anna P Schenck

Anna P. Schenck, PhD

11/26/2018

Date

Megin Nichols

Megin Nichols, DVM, MPH

26 Nov 2018

Date

ABSTRACT

Erin Frey: Antimicrobial Resistance and Zoonotic Outbreaks of *Salmonella enterica* in the United States, 2015-2018
(Under the direction of Anna P. Schenck)

The emergence of antimicrobial resistance worldwide has threatened the therapeutic viability of antimicrobial drugs. Non-typhoidal *Salmonella enterica* subspecies *enterica* sickens 1.2 million Americans annually and can be transmitted to people through food, water, animal contact, and animal environments. This paper represents the first description of the epidemiology of multistate zoonotic outbreaks of non-typhoidal *Salmonella* in the US and their representative patterns of antimicrobial susceptibility and resistance of patient isolates. It evaluates the rate of concordance between traditional phenotypic and newer genotypic predicted antimicrobial resistance patterns to in the context of *Salmonella* surveillance and treatment in the US that rely on the cooperation between local, state and federal public health organizations. Given the complex public health challenges public health leaders are urged to take the role of chief health strategists by using transformational leadership and systems thinking to engage stakeholders in the One-Health effort to combat zoonotic salmonellosis and antimicrobial resistance.

To my husband and my son, I could not have done this without your love and support.

ACKNOWLEDGEMENTS

To Anna Schenck in Public Health Leadership Department in the UNC Gillings School of Global Public Health for her review and editing of this document and her support throughout my MPH program. To the staff in the CDC's Division of Foodborne, Waterborne and Environmental Diseases, particularly, Megin Nichols, my preceptor and Enteric Zoonoses Activity team lead, who with Louise Francois-Watkins on the NARMS Team, created the original research idea and made it possible for me to work onsite with the CDC team on this project. Their help with providing background material on NARMS and outbreak surveillance in the United States as well as interpreting outbreak data and resistance profiles was invaluable.

To Lia Koski and Lauren Stevenson, who helped to procure, compile, and organize the original outbreak data, Zac Schneider, who linked the outbreak line listings to the resistance data in the PulseNet system, Jessica Chen in the NARMS lab, who screened whole genome sequences for predicted resistance determinants in PulseNet, Meseret Birhane, who performed data collection and analysis, Heather Carleton, who helped to interpret the whole genome sequencing tree descriptions, and Kaitlin Tagg, who verified and reviewed discordant resistance profiles of outbreak isolates, all of your help was essential in the completion of this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APHIS	Animal Plant Health Inspection Service
AST	Antimicrobial Susceptibility Testing
AVMA	American Veterinary Medical Association
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DFWED	Division of Foodborne, Waterborne and Environmental Diseases
EDEB	Enteric Disease Epidemiology Branch
EZA	Enteric Zoonoses Activity
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
hqSNP	High-quality Single Nucleotide Polymorphism
IAP2	International Association for Public Participations
NARMS	National Antimicrobial Resistance Monitoring System
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
NNDSS	Nationally Notifiable Disease Surveillance System
NORS	National Outbreak Response System
OIE	World Organisation for Animal Health
ORPB	Outbreak and Response Prevention Brach
PFGE	Pulsed-field Gel Electrophoresis
PulseNet	National Molecular Subtyping Network for Foodborne Disease Surveillance

Pred-R	Predicted Resistance
USDA	United States Department of Agriculture
wgMLST	Whole Genome Multilocus Sequence Type
WGS	Whole Genome Sequencing
WHO	World Health Organization

CHAPTER 1: MASTER'S PAPER FORMAT

The focus of this paper is to describe the epidemiology of zoonotic outbreaks of non-typhoidal *Salmonella enterica* in the United States between 2015 and 2018. This analysis includes the descriptive epidemiology of those identified in outbreaks and the characterization of the resistance pattern of the bacterial isolates including comparing phenotypic resistance patterns from antimicrobial susceptibility testing and predicted resistance from whole genome sequencing. The paper is organized into the following three chapters.

Zoonotic Outbreaks of Salmonella Enterica and Surveillance Systems in the United States: Background

Chapter 2 is an introduction to the importance of antimicrobial drugs in treating human and animal illness, the burden of zoonotic salmonellosis in the United States, the context of state and federal surveillance programs for non-typhoidal *Salmonella*, and mechanisms for and patterns of antimicrobial resistance in a local and global context.

Antimicrobial Resistance and Zoonotic Outbreaks of Salmonella Enterica in the United States, 2015 – 2018

Chapter 3 is the output of my master's practicum with colleagues at the Centers for Disease Control and Prevention in the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) in the Division of Foodborne, Waterborne and Environmental Diseases (DFWED) in the Enteric Disease Epidemiology Branch (EDEB) and Outbreak Response and Prevention Branch (ORPB). This section will be reviewed and cleared by staff at CDC for submission to a relevant microbiology journal in order to

inform current surveillance protocols during multi-state outbreaks of zoonotic disease including protocols for sample submission and testing for antimicrobial resistance.

A Public Health Practitioner's Role as Chief Health Strategist to Combat Antimicrobial Resistance and Zoonotic Outbreaks of Salmonella Enterica in the United States

Finally, Chapter 4 describes opportunities to apply public health leadership concepts to the topic of enteric zoonotic disease and antimicrobial resistance including using a systems thinking approach to combatting zoonotic outbreaks of non-typhoidal *Salmonella enterica* and promoting antimicrobial stewardship in the United States. It explores ways to identify stakeholders at multiple levels of society and to describe their role level of interest and influence in the problem as well as their relative support and contribution to success public health interventions. The role of the public health professional as the chief health strategist to promote antimicrobial stewardship and outbreak prevention and value of transformational leadership and leadership at all levels is explored to achieve these goals. These concepts are related to my personal journey as a veterinarian and public health professional and to my career goals and objectives.

CHAPTER 2: ZOONOTIC OUTBREAKS OF SALMONELLA ENTERICA AND SURVEILLANCE SYSTEMS IN THE UNITED STATES

Introduction

The discovery of antibiotics has been lauded as one the ten greatest public health achievements of the 20th century (CDC, 1999). With their discovery previously fatal infections from bacteria such as *Staphylococcus* spp. and *Streptococcus* spp. were no longer incurable. Unfortunately, these once seemingly miraculous drugs are in danger of becoming obsolete since the prevalence of antimicrobial resistance is increasing locally and globally. The use of antimicrobial drugs causes selection pressure on bacterial populations, whether pathogenic or commensal microbiota, that can lead to the development or uncovering of resistant subpopulations of bacteria. In the instance when a bacterial strain or subpopulation stops being susceptible to an antimicrobial drug, it is considered to have acquired resistance (UMN, AMRLS, 2018). This is distinct from the innate or inherent resistance that bacterial species may have to certain antimicrobial drugs or drug classes, referred to as intrinsic resistance. While clinicians are accustomed to taking into account intrinsic resistance when choosing antimicrobial drugs for their patients' infection, the possibility of acquired antimicrobial resistance makes treatment failure more likely and increases the need for antimicrobial susceptibility testing as a part of diagnostic and treatment plans.

Antimicrobial use, even when judicious or appropriate, is a driver of the increasing prevalence of antimicrobial resistant strains of bacteria, and even as more

antimicrobial drugs have been discovered, resistance to those antimicrobials has developed (CDC, 2013, *AR Threats*; CDC, 2018, *What is Resistance?*) The World Health Organization (WHO) has called antimicrobial resistance, “one of the biggest threats to global health, food security, and development today,” and cautions that resistant bacterial strains can lead to “longer hospital stays, higher medical costs and increased mortality” (WHO, Antibiotic Resistance, 2018). The current number of antimicrobial drugs under development has slowed, and according to the WHO “none of them are expected to be effective against the most dangerous forms of antibiotic-resistant bacteria” (WHO, Antibiotic Resistance, 2018). This has threatened to stall progress on the control of infectious diseases such as tuberculosis and raises concerns that routine surgery and cancer chemotherapy may no longer be successful (WHO, Antimicrobial Resistance, 2018).

2.1 Salmonella enterica subspecies enterica

Salmonella enterica subspecies *enterica* is a gram-negative, rod-shaped bacillus that is further subdivided into serotypes that cause typhoid fever and those that do not, called non-typhoidal *Salmonella.* The focus of this paper is on the latter, non-typhoidal *Salmonella*, which is transmitted by the fecal-oral route and typically causes acute diarrhea with or without blood, abdominal cramps, fever, and possibly nausea, vomiting, or headache. More serious outcomes of *Salmonella* infection include bacteremia, meningitis, osteomyelitis, septic arthritis, and death (CDC, *Information for Healthcare Providers*, 2018). Every year in the US there are an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths caused by non-typhoidal *Salmonella*. 1.1 million of the estimated 1.2 million illnesses are acquired in the US, and of these, 1

million (or an estimated 15.2 illnesses per 100,000 people) result from exposure to a contaminated food source (CDC, *Information for Healthcare Providers*, 2018; CDC, *Q&A*, 2018; Scallan et al., 2011). The remaining vehicles for transmission include contact with contaminated pet food, infected animals or animal surroundings including enclosures (e.g., cage, tank, pen) or environments (e.g., barn or kennel surfaces, railings, straw) (CDC, *Information for Healthcare Providers*, 2018).

2.2 Salmonella Reporting – Public Health Surveillance

National surveillance for *Salmonella* relies on several passive and active forms of reporting. Although salmonellosis has been part of the National Notifiable Disease Reporting System (NNDSS) since 1944, in 2018, the case definition for reporting *Salmonella* was split into two categories to differentiate Salmonellosis (excluding paratyphoid fever and typhoid fever) and Paratyphoid fever (caused by *Salmonella* serotypes Paratyphi A, Paratyphi B [tartrate negative] and Paratyphi C) (CDC, n.d., *Salmonella Case Definition 2017*; CDC, *Salmonella*, *Summary NNDSS*, n.d.). Clinicians voluntarily report cases as part of the NNDSS system and send isolates to public health laboratories that confirm, serotype and forward on unusual or untypable isolates to the CDC for further testing as part of the Laboratory-based Enteric Disease Surveillance System (LEDS) (CDC, *Salmonella* | *Surveillance*, 2018). To enhance epidemiological reporting from laboratory-confirmed cases of salmonellosis, the Foodborne Diseases Active Surveillance Network (FoodNet) conducts active surveillance using 10 sites nationally that cover an estimated 15% of the US population (CDC, *FoodNet*, 2016). The National Outbreak Reporting System (NORS) assists local, state and territorial health departments to report “enteric disease outbreaks caused by bacterial, viral,

parasitic, chemical, toxin, and unknown agents, as well as foodborne and waterborne outbreaks of non-enteric disease" that include non-typhoidal *Salmonella* regardless of whether it is spread through water, food, people, animals, or environmental contamination (CDC, *National Outbreak Reporting System (NORS)*, 2018). Even with these surveillance system underdiagnosis does occur, and in the case of foodborne outbreaks of non-typhoidal *Salmonella*, the true number of cases is estimated at 29.3 times the number diagnosed (Scallan et al., 2011).

2.3 Salmonella Outbreak Detection

The timeline from when a person is infected with *Salmonella* bacteria to when the person is determined to be part of an outbreak can take from two to four weeks. For a patient to be included in an outbreak case count requires action and resources (e.g., money, time and personnel) from patients, health care providers and public health laboratories. This process starts when a person develops symptoms of illness and proceeds to an exam by a health care professional, who submits a patient sample to the lab for bacterial testing. If the laboratory determines that the bacterial sample contains *Salmonella*, state regulations require that it be sent to a public health laboratory, such as any one of the 83 PulseNet laboratories across the country, where they undergo pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) to determine their genetic fingerprint for serotyping and DNA fingerprinting (CDC, *Timeline for Reporting*, 2015). These patterns are then uploaded to PulseNet, which is the molecular subtyping network for foodborne disease surveillance, and if two or more patterns match, CDC scientists evaluate the cluster to determine whether it constitutes an outbreak of disease (CDC, *PulseNet*, 2016). Since 2014 the CDC has supplemented

PFGE pattern testing in cluster investigations with WGS because it quantifies isolate relatedness more precisely using allele or single nucleotide polymorphism differences between isolates rather than the less discriminatory PFGE “fingerprint” band pattern.

If a patient meets the established outbreak case definition including clinical (e.g., diarrhea, abdominal pain, nausea and sometimes vomiting), laboratory (e.g., a culture independent test supports and culture confirms the presence of *Salmonella*), and epidemiologic criteria (e.g., person, place, time), they are added to the outbreak count (CDC, *Surveillance Case Definition | Salmonella*, 2017). Throughout this process health departments conduct hypothesis generating and hypothesis testing questionnaires, traceback and food and environmental testing to determine the source of the outbreak (CDC, *Outbreak Infographic*, n.d.). In the case of salmonellosis linked to animal outbreaks this might include testing animals (e.g., fecal or cecal samples) or their environment (e.g., turtle water, animal pens or bedding).

2.4 Enteric Zoonoses Activity in the Outbreak Response and Prevention Branch Division of Foodborne, Waterborne and Environmental Diseases

Within the CDC’s Outbreak Response and Prevention Branch (ORPB) of the Division of Foodborne, Waterborne and Environmental Diseases (DFWED), the Enteric Zoonoses Activity (EZA) team is responsible for conducting multistate enteric zoonoses outbreak investigations (CDC, *Enteric Zoonoses Activity*, 2018). When hypothesis-generating interviews from patients identified as part of an outbreak indicate a pattern of animal exposure that is greater than expected, outbreak response teams work to secure samples from animals and animal environments that people had contact with before becoming ill to enable comparisons with PFGE and/or WGS patterns from the ill people. Outbreaks linked to animals have resulted from contact with live poultry, reptiles (e.g.,

geckos, lizards, and snakes), dairy calves, and most recently guinea pigs (CDC, *Salmonella* | *Healthy Pets/Healthy* People, 2018).

For example, between 1990 and 2015, there were 57 outbreaks linked to *Salmonella* from baby poultry that included 2,885 illnesses, 450 hospitalizations, and five deaths (CDC, *Don't Play Chicken with Your Health*, n.d.). Children aged < five years typically get sick at higher rates than other groups, and young children, the elderly, and those with weakened immune systems, such as people undergoing chemotherapy, typically have higher rates of serious outcomes such as hospitalization or death. While antibiotics are not generally needed or recommended for mild cases of salmonellosis, they are sometimes used in severe cases or in the aforementioned vulnerable groups and include first line (i.e., ciprofloxacin (adults), azithromycin (children, pregnant women), or ceftriaxone) and alternative options (e.g., ampicillin, trimethoprim-sulfamethoxazole) (CDC, *Information for Healthcare* Providers, 2018; Shane et al. 2017).

2.5 Antimicrobial drug resistance threat from non-typhoidal Salmonella enterica

The CDC, in its 2013 Antimicrobial Resistance Threats Report, gave non-typhoidal *Salmonella* the middle threat level of *Serious*, defined as "significant antibiotic-resistant threats. For varying reasons (e.g., low or declining domestic incidence or reasonable availability of therapeutic agents), they are not considered urgent, but these threats will worsen and may become urgent without ongoing public health monitoring and prevention activities." (CDC, *AR Threats*, 2013)

In light of growing numbers of antimicrobial resistant strains of bacteria globally, in 1996 the National Antimicrobial Resistance Monitoring System (NARMS) began

tracking “changes in the antimicrobial susceptibility of certain enteric (intestinal) bacteria found in ill people (CDC), retail meats (FDA), and food animals (USDA) in the United States” (CDC, 2018, NARMS). Additional goals of the NARMS surveillance program include communicating findings to stakeholders in the US and abroad for the purpose of instituting preventive strategies, engaging in research on the “emergence, persistence, and spread of antimicrobial resistance,” and sharing data with the FDA that can be used to inform decisions about antimicrobial drug approval for animal use (Karp, 2017).

While NARMS focuses on resistance data, resistance information from NARMS is integrated with other national surveillance efforts. Isolates tested by NARMS may be linked using a common isolate identifier to records from PulseNet, NORS, and FoodNet (Karp, 2017). If an isolate is matched to an outbreak, the CDC links epidemiologic and laboratory data from PulseNet and NARMS to aid in outbreak investigation and response (Karp, 2017). Currently states are asked to submit a minimum of three representative isolates from each single-state outbreak to the CDC for NARMS testing (CDC, *NARMS Human Isolates Surveillance Report for 2015*; CDC, *EDLB Submission Table*, 2018). In addition to submitting every 20th sample of nontyphoidal *Salmonella* for surveillance, CDC requests that states submit additional isolates for multistate outbreaks; typically three to five isolates are requested per multistate outbreak, but additional isolates are requested when the epidemiology (e.g., subclusters of cases linked by common exposure) or molecular characteristics (e.g., PFGE patterns or WGS clades) indicate diversity within the outbreak (CDC, NARMS Human Isolates Surveillance Report for 2015).

2.6 Testing for antimicrobial drug resistance in non-typhoidal Salmonella enterica: Phenotypic and genotypic testing

Salmonella isolates cultured from patient samples (typically stool, urine, or blood) were previously tested using antimicrobial susceptibility testing (AST) to determine whether the bacterial strain had phenotypic resistance to a defined set of antimicrobial drugs. Antimicrobial susceptibility testing methods and interpretive criteria used by the CDC were developed through work with the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Karp, 2017). Of the 18 antibiotics from 12 drug classes that NARMS uses to test bacteria for resistance, the panel for testing *Salmonella* bacteria from 2015–2018 included 15 antibiotics, of which 13 were used throughout the entire period, one (ceftiofur) was included until 2015, and one (meropenem) was included beginning in 2016 (CDC, *NARMS Report 2015, Table 2*, 2018) (Table 1).

One challenge of traditional AST testing is the time (e.g., days) it takes to physically ship bacteria from state health departments to CDC, to grow bacteria in culture and to perform identification and susceptibility testing. With the newer method of WGS bacteria can be screened rapidly for genetic determinants of resistance and compared to strains from different sources for genetic relatedness (Karp, 2017). In 2015, the EZA was created at CDC and began requesting WGS of outbreak isolates in addition to the epidemiologic data from outbreak questionnaires and surveys. WGS can be used to detect resistance genes for antimicrobial drugs that are not part of the phenotypic resistance testing (i.e., AST panel) and can differentiate genetic mechanisms of resistance that lead to the same predicted resistance pattern (i.e., multiple genes or mutations can result in resistance to the same antimicrobial drug)

(McDermott, 2016). Having a whole genome sequence of an outbreak isolate strengthens the epidemiologic and laboratory evidence between outbreak cases and between samples from people, animals, foods, and the environment.

These data are compiled into a WGS “tree” that depicts the genomic relatedness of samples in an outbreak, either through high-quality single nucleotide polymorphism (hqSNP) or whole genome multilocus sequence typing (wgMLST) trees that show the number of SNP or allele differences between isolates, respectively. Because the data from past outbreaks remains in the PulseNet database, WGS trees also include historical samples from past outbreaks (whether human, animal, food, or environmental) to put current outbreaks in context (Karp, 2017).

One drawback of WGS is it relies on known resistance genes, which is why phenotypic testing of bacteria through AST will still be necessary to uncover new resistance patterns (Karp, 2017). Additionally, while AST demonstrates the expressed phenotypic resistance of an isolate (i.e., how it would respond to antimicrobials given to a patient), WGS detects the presence but not necessarily the expression of resistance genes (i.e., having genes that code for resistance does not guarantee that the bacteria will express those genes and induce resistance to that antimicrobial drug in the person or animal, from which it was isolated). Although WGS has been shown to predict antimicrobial resistance with a high degree of accuracy in non-typhoidal *Salmonella*, phenotypic resistance profiles derived from AST results are considered the gold standard when both types of testing are performed (McDermott et al., 2016).

Although the CDC currently requests three to five isolates per outbreak, with the increase in WGS and culture-independent testing (CIDT), it is vital to understand how

many outbreak samples and what type of testing (i.e., AST vs. WGS) are needed to characterize the etiological agent and resistance patterns of an outbreak. Given limited personnel and monetary resources and the demands on time for the health care professionals, laboratorians, and health department employees, the protocol for sample submission will likely be a trade off between a desire for the greatest accuracy and precision while using resources efficiently.

2.7 Significance of this paper

The convention for outbreak reporting whether foodborne or zoonotic is to post a summary on the CDC website and share it with the media. On occasion outbreaks findings are presented as reports in the CDC's Morbidity and Mortality Weekly (MMWR), or trends for one vehicle type are explored in more detail, such as live poultry-associated outbreaks from 1990–2014 (Robertson et al., 2018; Basler et al., 2016). This paper represents the first time outbreak data from multiple years and from multiple zoonotic vehicles has been compiled and analyzed to evaluate the epidemiology and health outcomes of patients and to characterize antimicrobial resistance patterns by vehicle type. Knowing which groups are most affected will allow public health professionals to shape messaging and outreach to those groups. In addition, being able to characterize outbreaks by vehicle type and expected resistance will aid in drafting diagnosis and treatment guidelines for frontline health care professionals.

It also is the first time that phenotypic and genotypic antimicrobial resistance patterns have been compared in a comprehensive manner across years and zoonotic vehicles to look for concordance between testing methodologies. By gaining an understanding which types of resistance discordance are common amongst zoonotic

outbreak, the NARMS team can update surveillance protocols and develop standardized guidance for testing and interpretation of results to aid health care professional in antimicrobial use decision-making.

CHAPTER 3: ANTIMICROBIAL RESISTANCE AND ZOONOTIC OUTBREAKS OF SALMONELLA ENTERICA IN THE UNITED STATES, 2015 – 2018

3.1 Abstract

The emergence of antimicrobial resistance worldwide has threatened the therapeutic viability of antimicrobial drugs. Non-typhoidal *Salmonella enterica* subspecies *enterica* sickens 1.2 million Americans annually and can be transmitted to people through food, water, animal contact and animal environments. This paper represents the first summary description of the epidemiology of multistate zoonotic outbreaks of non-typhoidal *Salmonella* in the US and their representative patterns of antimicrobial resistance in patient isolates. Thirty-two outbreaks (n=2691 patients) of zoonotic salmonellosis attributed to contact with live poultry, reptiles, dairy calves, and guinea pigs affected infants aged <1 month to adults aged >100 years and resulted in 638 hospitalizations and 6 deaths between 2015 and 2018. Outbreaks linked to contact with live poultry represented the majority (>85%) of outbreaks and patients over the study period. Thirty-eight percent (n=1030/2691) of patient isolates underwent traditional antimicrobial susceptibility testing and/or whole genome sequencing for predicted resistance, and 40 distinct patterns of antimicrobial resistance emerged. Of the isolates tested (n=1030),19.2% (n=198) demonstrated resistance to at least one antimicrobial drug, and 13.8% (n=142) were resistant to 3 or more antimicrobial drugs. Resistance to streptomycin, sulfisoxazole, tetracycline, and ampicillin were the most prevalent. Traditional phenotypic and genotypic predicted patterns of resistance agreed

with or were explained by differences in phenotypic and genotypic testing protocols in over 90% of patient isolates. These results will be used to inform national *Salmonella* surveillance protocols and to coordinate local, state and federal outbreak response.

3.2 Introduction

In the United States the National Antimicrobial Resistance Monitoring System (NARMS) was established to integrate surveillance and tracking of antimicrobial resistance patterns in enteric pathogens from people, retail meats, and animals. It is a collaboration of the Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and the United States Department of Agriculture (USDA) with cooperation and support from local, state, and territorial health departments who submit bacterial isolates. Health departments are asked to submit every 20th *Salmonella* isolate to CDC-NARMS for antimicrobial resistance testing as well as three to five representative isolates from each outbreak of the above pathogens. Samples submitted to NARMS by 54 state and large urban health departments undergo antimicrobial susceptibility testing (AST) by broth microdilution using a 14-drug Sensititre® panel (CDC, *NARMS Report 2015*, 2018). Previous internal assessments of NARMS data by CDC staff indicated that the current protocol of submitting three to five isolates per outbreak was generally sufficient to acquire an outbreak's major resistance profile.

The phenotypic resistance profile determined by AST is complemented by the predicted resistance from genetic profile of sequenced isolates submitted to PulseNet. Although PulseNet was originally envisioned and piloted in 1996 as a way to detect and respond to outbreaks of foodborne disease, bacterial isolates from ill persons have been linked to outbreaks caused by contact with animals or the environment (e.g.,

lakes, pools) (CDC, *FAQs PulseNet*, 2016; CDC, *Outbreak Detection*, 2016). Through partnerships with state and large urban health departments PulseNet performs whole genome sequencing (WGS) on outbreak isolates, and sequence differences are compared using whole genome multilocus sequence type (wgMLST) or high-quality single nucleotide polymorphism (hqSNP) analysis and displayed visually on a tree that depicts isolate relatedness and clustering into distinct clades. Isolates submitted to PulseNet can be compared via wgMLST or hqSNP analysis to outbreak-associated isolates including those from human cases and implicated vehicles (e.g., food or animal origin). Outbreak isolates are typically selected for submission to NARMS before the outbreak investigation is complete for both single-state and multistate outbreaks. The selection of isolates for susceptibility testing is independent of the decision to create a WGS tree to aid the epidemiologic investigation. Therefore, isolates from a given outbreak may be selected for susceptibility testing without prior knowledge of their genetic relationship to other isolates in the outbreak. Susceptibility testing results from NARMS are linked to data in PulseNet at the level of the individual isolate once the results have laboratory approval. Susceptibility testing results are linked to epidemiologic data from each outbreak reported to NORS at the level of the outbreak after NARMS and NORS have completed data cleaning for the annual cycle (typically > 1 year after the conclusion of the outbreak).

Enteric pathogen isolates are evaluated for the presence of resistance determinants (i.e., genes and mutation) using a modified version of ResFinder software (BioNumerics), and these resistance determinants are used to predict the resistance of outbreak isolates to the same antimicrobial drugs that are on the phenotypic NARMS

AST panel. WGS has been shown to predict antimicrobial resistance with a high degree of accuracy in non-typhoidal *Salmonella* (McDermott et al., 2016). Although concordance between predicted and genotypic resistance patterns has been found human and retail meat isolates (McDermott et al, 2016), no such study has compared phenotypic resistance by AST with genotypic resistance from WGS for human isolates from zoonotic outbreaks of *Salmonella*.

Since 2015 when the CDC began requesting WGS for isolates from zoonotic outbreak of enteric disease (specifically non-typhoidal *Salmonella*), the proportion of samples with sequencing data has increased. Using existing epidemiologic, AST and WGS data from zoonotic outbreaks of non-typhoidal *Salmonella* that have occurred since widespread use of WGS methodology (2015 through 2018), we performed descriptive epidemiology of enteric zoonotic disease outbreaks and described antimicrobial resistance in zoonotic outbreaks and the frequency of resistance discordance.

3.3 Methods

Data and specimen collection

Epidemiologic data used in this analysis were collected during outbreak investigations to aid outbreak response. Records reside at both the CDC and state and large urban health departments since the latter usually interviews case-patients and submits certain elements of the resulting epidemiologic data to the CDC. To fulfill requirements of the National Notifiable Diseases Surveillance System (NNDSS), *Salmonella* isolates were submitted to state or large urban health departments, and a subset of these isolates (3–5 per outbreak) was submitted to the CDC for antimicrobial

susceptibility testing (AST), whole genome sequencing (WGS), or both. All specimens were identified with a corresponding isolate ID, which is a coded variable that protects personal identifiers. The original health department, from which the sample originated, has access to the key to personal identifiers.

Data and specimen disposition

Isolates previously submitted to CDC-NARMS for AST and/or WGS testing were frozen and will be stored indefinitely at CDC according to standard NARMS protocol. Isolates previously submitted and sequenced at local and state health departments will be subject to individual site disposition protocol, which usually entails storage for months to years but not indefinitely. Whole genome sequencing data will be stored in the PulseNet database according to PulseNet protocol. Finally epidemiologic data will be stored indefinitely in the CDC's outbreak database according to ORPB protocol. A coded isolate ID to protect personal identifiers will identify specimens, and data will be presented in aggregate to avoid discerning individual-level patient information

Outbreak Identification

Zoonotic outbreaks of enteric disease with a known or suspected vehicle from 2015–2018 were identified from the CDC's Enteric Zoonoses Activity (EZA) team, a subset of ORPB. Although other outbreaks of zoonotic disease covered by ORPB and NARMS (e.g., *Campylobacter jejuni* in puppies in 2017) were noted during the time period, only outbreaks of non-typhoidal *Salmonella* were included in this analysis because it was the primary zoonotic outbreak pathogen identified and because NARMS submission protocols were expected to yield adequate data for analysis of both descriptive epidemiology and resistance patterns.

Some outbreak investigations involved more than one *Salmonella* serotype but were investigated under a single outbreak code due to the cases' shared exposure to a common transmission vehicle (i.e., animal type); however, distinct serotypes are neither closely genetically related nor expected to share a common resistance profile. Prior to 2017, outbreaks of live poultry-associated salmonellosis were assigned a unique outbreak code based on the *Salmonella* serotype of the cluster; however, beginning in 2017, all live poultry outbreaks from the calendar year were grouped under one outbreak code due to the common outbreak vehicle. Given that WGS trees were constructed from individual serotypes and even subsets of a serotype based on the predominant PFGE pattern(s), live poultry outbreaks for the entirety of the study period were treated as distinct outbreaks based on the serotype/PFGE pattern combination regardless of the outbreak code used to identify them. Despite having one outbreak code, an outbreak of turtle-associated salmonellosis from 2015 was also treated as two separate outbreaks because two serotypes were identified, of which only one serotype had WGS analysis.

Laboratory Analysis

Completed outbreak investigations from 2015–2018 with existing WGS trees were identified, and individual isolates were classified according to their position on the tree (e.g., main clade, clade B, clade C, outlier) when included. Isolates were screened for the presence of resistance determinants using ResFinder 3.0 and for the presence of plasmids using PlasmidFinder 3.0 (Center for Genomic Epidemiology, DTU). Resistance patterns were predicted for each isolate based on the presence of resistance determinants according to established protocols and criteria (McDermott et

al., 2016). When both AST and WGS were performed on the same isolate, the predicted resistance (Pred-R) pattern from WGS was compared to the phenotypic resistance pattern from AST to ensure that no information was lost with updated sampling protocols. When phenotypic and genotypic resistance patterns were discordant for an antimicrobial drug on the standard NARMS panel, phenotypic AST results were given precedence over Pred-R from WGS.

Epidemiologic Analysis

Outbreak records from the ORPB database were obtained for each outbreak identified as above. Since reporting and grouping of data were not standardized during this period, and since outbreak questionnaires varied based on the suspected or confirmed vehicle, a core set of epidemiologic variables common to all outbreaks was determined. These include age (minimum, maximum, and median), gender (male/female), race (white/black/other), ethnicity (Hispanic/Latino: yes/no), hospitalization (yes/no), died (yes/no), and exposure to the implicated outbreak vehicle (yes/no). Data were considered missing if the record was blank or had the answers of 'don't know,' 'unknown,' or 'no answer.' Where possible, missing data from compiled multiple line listings for outbreak epidemiology was supplemented from demographic information derived from matched PulseNet records, which were only available for a subset of patients whose isolates were submitted for AST or WGS analysis. Epidemiology of the complete set of outbreaks was compared to a subset of outbreaks with at least 25% of outbreak isolates having WGS and a further subset that had WGS for 10 or more isolates totaling at least 25% of outbreak isolates. These cut-offs were chosen to reduce the likelihood of Type II error and increase the likelihood that the

subset of case isolates within an outbreak with WGS and Pred-R data would be representative of the whole outbreak case set.

Descriptive epidemiologic methods were used to characterize overall resistance profiles of each outbreak including resistance patterns, resistance to individual antimicrobial drugs or drug classes, and frequency of resistance by outbreak vehicle type, and resistance discordance was examined at the isolate level. For each outbreak, epidemiologic variables (as noted above) were compared between the full set of outbreak isolates and the subset of isolates, which had been analyzed for resistance using phenotypic (AST) and/or genotypic (WGS) predicted resistance testing.

Outbreak isolates were analyzed for resistance to the 13 antimicrobials in nine CLSI drug classes that were part of the standard NARMS AST panel throughout the entire study period (2015–2018). These include aminoglycosides (gentamicin, streptomycin), beta-lactams/beta-lactamase inhibitor combinations (amoxicillin-clavulanic acid), cephems (ceftriaxone, cefoxitin), folate pathway inhibitors (sulfisoxazole, trimethoprim-sulfamethoxazole), penicillin (ampicillin), phenicols (chloramphenicol), quinolones (ciprofloxacin, nalidixic acid), and tetracyclines (tetracycline) (Table 1). Ceftiofur and meropenem were excluded because they were not included over the complete study period. Ceftiofur was included in the NARMS AST panel until 2015, and meropenem was added to the AST panel in 2016. An additional aminoglycoside antibiotic, kanamycin, was included in the analysis because genetic patterns of predicted resistance are included in Pred-R testing.

Outbreak resistance patterns were further compared based on the implicated animal vehicle (i.e., live poultry, reptile, dairy calves, guinea pigs). Because of the small

size of outbreaks linked to reptiles (11–102 cases) and the small number of isolates sequenced in each outbreak (0–49), for the purpose of comparison by resistance patterns, all reptile-associated outbreaks (i.e., gecko, turtle, snake) were compiled into one category. This is in keeping with how public health interventions are described by the CDC and other regarding reptiles and amphibians. Although dairy calves and guinea pigs are both mammals, their husbandry (on farm vs. in homes or schools) is different enough to warrant that these outbreaks be kept as distinct categories. Epidemiologic variables were compared between cases with resistant and susceptible isolates. The analysis was also stratified by the implicated vehicle or source type. Finally, antimicrobial resistance patterns for isolates within each outbreak were examined in regards to their position on the associated WGS tree (i.e., wgMLST or hqSNP) where available.

3.4 Results

Outbreak Epidemiology

From 2015 to 2018 there were 33 multistate zoonotic outbreaks of enteric non-typhoidal *Salmonella* investigated by the CDC Enteric Zoonoses Activity. One outbreak traced to turtles in 2016 was excluded from further analysis because it contained four distinct serotypes/PFGE patterns, a small number of isolates (n=6), of which only one sample had whole genome sequencing performed. From the remaining 32 outbreaks, 23 outbreaks or 85.0% (2287/2691) of cases were linked to live poultry, seven outbreaks or 12.6% (338/2691) of cases to reptiles (i.e., geckos, turtles, and snakes), one outbreak or 2.1% (26/2691) of cases to dairy calves, and one outbreak or 0.4% (10/2691) to guinea pigs. Of the 2691 outbreak cases, 37.4% (n=1008) of isolates had

whole genome sequencing results, and 38.3% (n=1030) had either AST or WGS performed to look for antimicrobial resistance.

Salmonella testing was performed on blood, urine and stool samples. From 64.5% to 100% of bacterial isolates were obtained through human stool samples compared to blood (0–22.4%), urine (0–16.7%), and other (0–9.1%). Other sample sources included rectal swab (n=7), wound (n=7), other (n=5), isolated organisms (n=3), and 1 each of abscess, bone, gallbladder, peritoneal fluid, rectum, sputum, swab and tissue.

Age data were available for between 91.7% and 100% of patients within each outbreak. 46.9% of outbreaks had age data for all patients, and all outbreaks had age data for more than 66% of their respective patients. Patients ranged from infants aged <1 month to a maximum of 106 years. At the outbreak level, patient age ranged from infants aged <1 month to one year, maximum age from 53 to 106 years, and median patient age as low as one year and as high as 56 years.

Data on patient gender (male/female) were available for 90.0%–100% of patients within each outbreak. Complete gender data was available in 28.1% of outbreaks, and all outbreaks had data for more than 66% of their respective cases. From 41.5% to 81.8% of outbreak patients were female with women representing the majority in 65.6% (21/32) of outbreaks.

Race and ethnicity data were only available from 34% (11/32) of outbreaks, in which race was recorded for 5.6%–70.0% of patients, and ethnicity was recorded for 7.1–70.0% of patients. No outbreaks had complete race or ethnicity data. For outbreaks in which racial data were available, 45% (5/11) of outbreaks had racial data in less than

33% of cases, and 18% (2/11) of outbreaks had racial data in more than 66% of cases. For outbreaks where race was reported, 37.5 to 100.0% of cases were white and 0–50.0% of cases were Black/African American. The remaining cases were from other racial groups including American Indian/Alaska Native (n=2), Asian (n=4), Hispanic (n=1), Latino (n=1), other (n=6), and Yemeni (n=1) (total = 15/177 available). Of outbreaks where ethnicity data were available, 36% (4/11) of outbreaks had ethnicity data in less than 33% of cases, and 9% (1/11) of outbreaks had ethnicity data in more than 66% of cases. For outbreaks from which ethnicity was available (11/32 outbreaks; 221 cases) from 0–68.8% of cases were of Hispanic/Latino descent.

Hospitalization and death data were available for 31 of 32 of outbreaks and from 56.5–100% of cases depending on the outbreak. Complete data on hospitalization and death were available from one outbreak each (3.1% of outbreaks). Reported health outcomes (hospitalization and death) were available for greater than 66% of case isolates in 90.6% of outbreaks. A total of 638 people were hospitalized, and the proportion of hospitalization at the outbreak level varied from 12.5%–55.6%. Zero deaths were reported in 81.3% (26/31) of outbreaks, one death was reported in 12.9% (4/31) of outbreaks, and two deaths were reported in the remaining outbreak for a total of 6 deaths reported overall. In the outbreak with two deaths, this resulted in an over case fatality rate of 9.1%.

Data on case contact with the implicated animal were available for 31 of 32 outbreaks. Complete exposure data was available in only one outbreak, but 81.3% of outbreaks had exposure data in at least 66% of case isolates. In one outbreak 9.5% of

cases had known contact with the vehicle, and in the remaining 30 outbreaks, 33.3% to 100% of cases reported contact with the vehicle before becoming ill.

Comparison to subsets based on proportion of outbreak isolates with WGS

Eighteen outbreaks had WGS results for at least 25% of samples, of which 10 outbreaks or 78.8% (1121/1423) were linked to live poultry, six outbreaks or 16.6% (236/1423) to reptiles, one outbreak or 3.9% (56/1423) to dairy calves and one outbreak or 0.7% (10/1423) to guinea pigs (Table 2). 62.4% (888/1423) of isolates had AST and/or WGS results. Of the 16 outbreaks that had WGS for at least 25% of samples and had 10 or more isolates sequenced, nine outbreaks or 79.9% (1110/1390) of cases were linked to live poultry, five outbreaks or 15.4% (214/1390) of cases to reptiles, one outbreak or 4.0% (56/1390) of cases to dairy calves and one outbreak or 0.7% (10/1390) to guinea pigs (Table 2). 62.9% (874/1390) had AST and/or WGS results.

When comparing the epidemiology of all outbreaks to the above subsets with >25% WGS (n=18 outbreaks) and ≥ 10 isolates and >25% with WGS (n=16 outbreaks), the only percentages that differed between the groups (32, 18, 16) were the maximum age (106 years, 101 years, 101 years), maximum % hospitalized (55.6%, 55.6%, 47.6%), maximum % exposed to the vehicle (100%, 88.9%, 88.9%), minimum data availability for hospitalization (56.5%, 61.1%, 61.1%) and death (56.5%, 61.1%, 61.1%) and maximum data availability for hospitalization (100%, 94.7%, 94.7%) and death (100%, 94.7%, 94.7%) (Table 3). Because epidemiologic variables were identical or very similar between the complete group and subsets, the full data set was reviewed for antimicrobial resistance patterns.

Epidemiologic Comparison between full case data to subset of isolates with antimicrobial resistance data

For the outbreaks in which more than 25% of the isolates were sequenced for predicted (genotypic) resistance (n=18), the subset of patients with resistance data within each outbreak were not substantively different from the full patient set. All outbreak minimum ages were within 1 year (67% were identical), and 89% of maximum and median ages were with 10 years of each other (67% and 17% were identical). Outbreak level epidemiologic variables were within 10 percentage points in 89% of outbreaks for the percent female, hospitalized, and exposed. 94% of outbreaks had identical rates of death between the full set and subset with resistance data.

Of the remaining 14 outbreaks that did not meet that 25% sequenced threshold, a further eight outbreaks had whole genome sequencing performed on 10–23% of cases representing between three and 30 isolates. Between the full set and the subset with resistance data outbreak level minimum ages were within one year for 75% of outbreaks (three were identical), maximum ages were within 10 years for 50% of outbreaks, and median ages were within 10 years for 88% of outbreaks. Outbreak level epidemiologic variables were within 10 percentage points of each other for female in 38% of outbreaks, hospitalization in 38% of outbreaks, death in 100% of outbreaks, and exposure in 50% of outbreaks.

Due to the minimal data available for race and ethnicity, these variables were not compared or analyzed between the complete outbreak epidemiology and the subset with resistance data. Even though outbreaks with less than 25% of isolates sequenced were not as representative of the whole as the outbreaks with a larger percentage with WGS, we evaluated the overall resistance patterns of all the outbreaks to get the most

data on resistance patterns and resistance concordance between AST and WGS results. These limitations were taken into account when interpreting the resistance patterns.

Descriptive Epidemiology of Antimicrobial Resistance

From the 32 outbreaks associated with zoonotic salmonellosis 85.0% of cases were from outbreaks linked to live poultry, 12.6% of cases were from reptile-associated outbreaks, 2.1% of cases were from dairy calf-associated outbreaks, and 0.4% of cases were from guinea pig-associated outbreaks (Table 4; Figure 1). Of the 16 outbreaks where at least one isolate was found to have antimicrobial resistance, 11 were associated with live poultry (n=1522, 86.8% of cases), three with reptiles (n=166, 9.5% of cases), one with dairy calves (n=56 cases, 3.2% of cases), and one with guinea pigs (n=10, 0.6% of cases) (Figure 1). Of the 10 outbreaks (n=599) where no isolates were found to have antimicrobial resistance, seven were associated with live poultry (n=529, 88.3% of cases) and three were associated with reptiles (n=70, 11.7% of cases). Finally of the 6 outbreaks (n=338) where antimicrobial susceptibility testing was not available, five were associated with live poultry (n=236, 69.8% of cases) and one with reptiles (n=102, 30.2% of cases).

Salmonella serotypes represented in outbreaks with documented resistance included Braenderup, Enteritidis, Hadar, Infantis, I 4,[5],12:I, Indiana, Litchfield, Mbandaka and Typhimurium in live poultry, Heidelberg in dairy calves, Agbeni, Mbandaka, and Paratyphi B Var. L(+) Tartrate in reptiles, and Enteritidis in guinea pigs. *Salmonella* serotypes in outbreaks with no documented resistance included Enteritidis,

Indiana, Infantis, Mbandaka, Muenchen, and Muenster in live poultry-associated outbreaks and Muenchen, Pomona, and Fluntern in reptile-associated outbreaks.

Of the 1030 isolated tested for antimicrobial resistance with AST or Pred-R, 19.2% (n=198) were resistant to at least one antimicrobial agent. Isolates from live poultry-associated outbreaks made up 82.2% (n=847) of isolates tested and 71.7% (n=142) of isolates with resistance; those from reptile-associated outbreaks made up 11.4% (n=117) of isolates tested and 4.0% (n=10) of resistant isolates; and guinea pig isolates comprised 1.0% (n=10) of isolates tested and 0.5% (n=1) of antimicrobial resistant isolates. In contrast isolates from dairy calf outbreaks were overrepresented since they included 4.7% (n=48) of isolates tested and 24.1% (n=47) of isolates with antimicrobial resistance. Overall 16.4% (139/847) of isolates from live poultry-associated outbreaks, 6.8% (8/117) of isolates from reptile-associated outbreak, 97.9% (47/48) of isolates from the dairy calf-associated outbreak, and 10.0% (1/10) of isolates from the guinea pig-associated outbreak had documented resistance to at least one antimicrobial agent (Table 4).

For each of the 13 antimicrobial drugs included in the NARMS AST panel between 2015-2018, resistance to each drug was noted in four to eleven outbreaks (median = 8) (Table 5). Of the 16 outbreaks where any type of resistance was documented, resistance to azithromycin was noted in the smallest number of outbreaks (4/16, 25%) and resistance to Streptomycin was noted in the most number of outbreaks (11/16,68.8%). Two antimicrobial drugs, for which there are known resistance genes, and which were also found in outbreak WGS data, were fosfomycin (n=1 outbreak) and kanamycin (n=2 outbreaks). In the case of the outbreak in which fosfomycin resistance

was predicted from WGS, AST results showed no phenotypic resistance because fosfomycin is not part of the standard NARMS panel.

For the 16 outbreaks and 198 isolates demonstrating resistance to at least one antimicrobial drug, there were 40 unique patterns of resistance to between one and eleven antimicrobial drugs. The most common patterns were ACSSuTAuCxFoxCip(I)Kan (n=30, 15.4% from three outbreaks), SSuGen (n=26, 13.3% from 10 outbreaks), T (n=23, 11.8% from 10 outbreaks), AAuCxFox (n=22, 11.3% from 12 outbreaks), and SSuTGen (n=17, 8.7% from 8 outbreaks) (Table 6). The remaining 35 patterns were seen in one (0.5%) to eight (4.1%) isolates. All but one pattern (NalCip = nalidixic acid, ciprofloxacin), which was found in both live poultry and reptile-associated outbreaks, were unique to the implicated vehicle. Live poultry-associated outbreaks included 28 unique resistance patterns, reptile-associated included four, dairy calf-associated included nine, and guinea pig-associated included only one resistance pattern (Table 6). 71.7% of isolates with documented resistance (13.8% of isolates tested) demonstrated multidrug resistance (i.e., defined as resistance to three or more antimicrobial drugs).

When broken down by frequency of resistance to each antimicrobial drug, resistance to streptomycin (S) was the most prevalent among isolates with resistance (126/198, 63.6%) followed by sulfisoxazole (Su) (117/198, 59.1%), tetracycline (109/198, 55.1%), and ampicillin (88/198, 44.4%) (Figure 3.3). The antimicrobial drug with the lowest prevalence of resistance was azithromycin (2% of isolates with resistance) found only in live poultry and reptile outbreaks. When resistance to individual antimicrobials was broken out by the implicated vehicle, outbreaks linked to

farm animals (i.e., live poultry and dairy calves) show resistance to 12 and 13 antimicrobial drugs (including kanamycin) while outbreaks linked to pets (i.e., reptile and guinea pig) show resistance to nine and three antimicrobial drugs respectively.

The outbreak involving dairy calves stands out from the other outbreak vehicles in having isolates with both a diversity of resistance (12 different antimicrobial drugs, eight of nine CLSI Drug Classes) as well as the high prevalence of resistance (over 70%) to 10 antimicrobial drugs (Figure 3.3). All resistant isolates (n=47) were resistant to 4 antibiotics (i.e., streptomycin, sulfisoxazole, chloramphenicol, and tetracycline) and 46 of 47 were resistant to a further 4 antibiotics (ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and cefoxitin), Although outbreak isolates were all *Salmonella* serotype Heidelberg, there were six distinct PFGE patterns with resistance patterns in the outbreak compared to live poultry outbreaks, of which seven of eleven outbreaks with antimicrobial resistance had only one PFGE pattern for the resistant isolates.

Resistant isolates from live poultry outbreaks showed the highest prevalence of resistance to streptomycin (54.7%), sulfisoxazole (48.9%), tetracycline (43.9%) and gentamicin (32.4%). In contrast, resistance among reptile outbreak isolates was the highest for ciprofloxacin (75%) and nalidixic acid (75%). The single isolate with resistance from the outbreak associated with guinea pigs showed resistance to streptomycin, sulfisoxazole, and trimethoprim-sulfamethoxazole. While resistance to 11 of the 14 possible antimicrobials was found in isolates three of the four outbreak vehicle types, resistance to kanamycin and azithromycin was found only in live poultry and reptile-associated outbreaks, and resistance to gentamicin was only seen in live poultry outbreaks (Figure 3.3).

When the epidemiology of the resistant isolates was compared to the non-resistant isolates, the maximum (89 yrs., 101 yrs.) and median (20 yrs., 25 yrs.) patient age was lower in cases with resistant isolates, and the percent hospitalized and with known exposure was higher in cases with resistant isolates (32.2%, 27.8% and 68.5% vs. 56.0%) (Table 7). A lower proportion of resistant isolates were from blood (2.6% vs. 4.9%) and from females (52.7% vs. 55.5%). Although race and ethnicity differed, the number with available data made it impossible to compare the two groups.

When comparing cases of live poultry-associated salmonellosis with resistant isolates (n=142) to those with non-resistant isolates (n=713), those with resistant isolates had lower maximum (83 yrs., 101 yrs.) and median (26.0 yrs., 28.0 yrs.) ages, a lower proportion of females (49.6%, 55.4%), and a higher rate of hospitalization (33.3%, 25.2%) and reported exposure to the vehicle (69.4%, 56.3%) (Table 8). In reptile-associated outbreaks of salmonellosis, cases with resistant isolates (n=8) had a higher proportion of females (87.5%, 57.5%), a lower maximum (40 yrs., 100 yrs.) and median (9.0 yrs., 13 yrs.) age, a lower hospitalization rate (0%, 46.4%), and a higher rate of exposure to the vehicle (80.0% vs. 52.1%) than cases with non-resistant isolates (n=109). No persons with resistant isolates died compared to one person with a non-resistant isolate each for a live poultry and reptile-associated outbreak. A similar comparison between resistant and susceptible isolates was not possible for guinea pigs and dairy calves because there was only one resistant isolate from the guinea pig-associated outbreak and only one susceptible isolate from the dairy calf-associated outbreak.

Whole Genome Sequencing Trees

The NARMS lab created WGS trees for 26 of 32 outbreaks (i.e., 24 hqSNP trees, 2 wgMLST trees). In addition, three samples from the 2015 outbreak of *Salmonella* Hadar were included on the 2016 hqSNP tree of *Salmonella* Hadar because they were highly related to the 2016 outbreak strains. In some WGS trees, antimicrobial-resistant isolates were spread across clades, such as in the 2015–2017 outbreak of *Salmonella* Heidelberg linked to dairy calves that had resistant isolates in both main clades. Clade A isolates were 0–21 SNPs apart, and they were 15–39 SNPs apart from Clade B, which included isolates 2–13 SNPs apart. In contrast, the 2017 live poultry-associated outbreak of *Salmonella* Hadar, which had five clades, showed antimicrobial-resistant isolates limited to clade E or after.

Of the 198 isolates for which resistance was documented through phenotypic and genotypic testing, 44% (n=87) were included on the trees, and 56% (n=111) were not included on the trees. During the *Salmonella* Litchfield outbreak associated with live poultry in 2017, no WGS tree was requested; however, sequencing and resistance data were available from patient isolates from this outbreak.

Resistance Concordance

Of the 116 samples with both AST and WGS results, 78% had identical phenotypic and genotypic resistance profiles. The remaining 22% (n=26) of isolates were found to have discordant antimicrobial resistance patterns for one (n=17), three (n=5), four (n=3), or five antimicrobial drugs (n=1) that included amoxicillin-clavulanic acid, azithromycin, chloramphenicol, fosfomycin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Since discordance

occurred for more than one antimicrobial, an explanation was needed for each discrepancy. An expert biostatistician in the CDC NARMS verified the reason for discrepancies in 19 of these 26 isolates based on knowledge of AST panel versus WGS Pred-R protocols and established characteristics of resistance determinance genes bringing the rate of concordance up to 94% of isolates with both phenotypic and genotypic resistance testing (KT, personal communication). In 18 isolates the predicted resistance determinant found on WGS was not part of the standard NARMS AST panel at the time of testing (fosfomycin, n=10, kanamycin, n=8). For the remaining isolates antimicrobial resistance was documented on AST but not on the Pred-R because of the presence of the novel gene dfrA34 (trimethoprim-sulfamethoxazole, n=8 isolates), because the qnrB19 gene is not used to predict nalidixic acid resistance (n=6 isolates), and on Pred-R but not on AST because a mutation in the floR gene caused a nonfunctional gene product conferring no resistance to chloramphenicol (n=6 isolates). The remaining seven discrepancies, representing 6.4% (7/109) of isolates with both AST and WGS, have theorized reasons for discrepancies including a known streptomycin mismatch between AST and Pred-R results due to lower specificity and positive predictive value than other antibiotics (McDermott et al, 2016) (n=2), susceptibility testing at the edge of the susceptible bell curve (n=2), and the possibility of plasmid loss prior to testing or low coverage genes (n=3).

Discussion

Epidemiologic Trends

Regardless of vehicle type the minimum age in all outbreaks was aged <1 year, and in over 50% outbreaks, the minimum age was aged <1 month. Median ages was

seven months to 56 years, and in 28% of outbreaks (n=9), the median patient age was <10 years, which suggests that parents and caregivers of young children are appropriate targets for public health education and interventions (CDC, *Information for Health Care Professional and Laboratories*, 2018). Since outbreak level maximum age exceeded 65 years in 90% of outbreaks, the elderly should also be considered a vulnerable group for zoonotic salmonellosis. These two groups (infants and individuals aged >65 years) are known to be at increased risk of serious disease or complications from *Salmonella* infections (CDC, *Information for Health Care Providers*, 2018). Since the age demographic held true all animal vehicles of salmonellosis, public health messaging regarding vulnerable groups can be consistent for zoonotic salmonellosis in general.

Additionally, women were the majority of the patients in 66% of outbreaks, and they continued to represent the majority of cases even after stratifying by the outbreak vehicle. Although the 90% to 100% outbreak level data availability on gender made conclusions possible, the lack of consistent racial and ethnicity data made it difficult to draw any firm conclusions about overall trends; however, for the 34.4% of outbreaks (n=11/32) in which racial data were available, the majority of cases reported being white in all but 1 outbreak.

Data on health outcomes (i.e., hospitalization and death) were available for over 66% of patients in over 90% of outbreaks. Prevalence of hospitalization was as low as 12.5% and as high as 55.6% at the outbreak level, but at least 25% of patients were hospitalized in 72% of all outbreaks. When stratified by outbreak vehicle, reptile-associated (37.9% of cases) and dairy calf-associated (34.7% of cases) outbreaks saw

higher rates of hospitalization than outbreaks associated with live poultry (28.9%) and guinea pig (12.5%). This higher rate of hospitalization for reptile and dairy calf outbreaks held true for outbreaks in which AST and WGS were carried out (n=26) regardless of whether any isolates in the outbreak were found to have antimicrobial resistance.

Death occurred in 0.2% of the 2691 patients identified, of which five were in live poultry outbreaks and one was in reptile-associated outbreak representing 0.3% and 0.4% of cases respectively. Given the relatively small number of cases associated with dairy calves and guinea pigs compared to live poultry and reptiles, no conclusion about trend can be made from this data.

Extent and persistence of outbreaks and pattern of outbreaks by vehicle type

Outbreaks associated with live poultry contact represented the majority (85.0%, 2287/2691) of patients over the study period compared to reptiles (12.6%), dairy calves (2.1%) or guinea pigs (0.4%). This difference persisted for the subset of outbreaks that had at least 25% of isolates sequenced (18 outbreaks, 78.8% linked to live poultry) and those that had both more than 25% of isolates sequenced and a minimum of 10 isolates sequenced (16 outbreaks, 79.2% linked to live poultry). Although not recorded in this paper because the outbreak was ongoing during the data analysis phase, there was another outbreak of live poultry-associated salmonellosis in 2018 involving a further 334 cases, 56 hospitalizations, and zero deaths, and the outbreak investigation of *Salmonella* Heidelberg from 2015–2017 was also reopened in 2018 (CDC, *Multistate Outbreaks of Salmonella Infections Linked to Contact with Live Poultry*, 2018). These outbreaks were not included in this analysis because the data analysis including AST and WGS were not completed at the time of writing.

Antimicrobial Resistance

Resistance data were available for 38% of all outbreak isolates, and 18.7% of isolates tested for antimicrobial resistance through phenotypic and/or genotypic tests showed resistance to at least one antimicrobial drug. While some isolates were only resistant to one antimicrobial drug, 71.7% isolates with any resistance were resistant to three or more antimicrobial drugs, which is the NARMS convention for multi-drug resistance. The study data showed a higher prevalence of resistance to five or more antimicrobial drugs (5.8% of isolates) than was previously reported in NARMS data from foodborne *Salmonella* outbreaks (i.e., 5% of non-typhoidal *Salmonella* isolates) (CDC, 2013).

Although there were 40 unique patterns of antimicrobial resistance found in these outbreaks, isolates from live poultry outbreaks showed the most variability with 28 patterns from 11 outbreaks comprising eight *Salmonella* serotypes (Braenderup, Enteritidis, Hadar, I 4,[5],12:I, Indiana, Infantis, Litchfield, Mbandaka, and Typhimurium) compared to reptiles with three outbreaks, three serotypes (Agbeni, Paratyphi B Var. L(+) Tartrate, and Mbandaka), and four resistance patterns, and to dairy calves with one outbreak, one serotype (Heidelberg), and nine resistance patterns. No conclusions can be drawn from the pattern in guinea pigs since only one isolate showed resistance (serotype Enteritidis). Of these serotypes, Enteritidis, Indiana, Infantis, and Mbandaka were also seen in outbreaks where no resistance was documented; however, these outbreaks had lower overall proportions of antimicrobial susceptibility testing (i.e., 10.9% in Mbandaka, 10% for Enteritidis, 17.3% for Indiana from 2016) or low number of isolates tested (e.g., Infantis, 2017, n=12 tested). Because of the variety of resistance

patterns in and between outbreak vehicle types and the finding that some serotypes were found in outbreaks both with and without AMR, health care professionals cannot rely solely on the *Salmonella* serotype for antimicrobial drug choice.

When stratified by vehicle, only the combined resistance pattern of nalidixic acid and ciprofloxacin (i.e., NalCip) was seen in both live poultry-associated and reptile-associated outbreaks; however, since resistance to 11 of 14 antimicrobial drugs was seen from isolates in outbreaks associated with three different species types, multidrug resistance was a problem common to all the vehicle species in this study and should be suspected and investigated in any type of zoonotic *Salmonella enterica* outbreak.

Although most cases of salmonellosis do not require antibiotics, treatment is recommended for patients with risk factors (e.g., immune compromise, young children or the elderly) or those with severe infections (e.g., invasive disease, blood stream infections) (Shane et al., 2017). In these cases, first line treatment includes ciprofloxacin, ceftriaxone and azithromycin and their alternatives ampicillin and trimethoprim-sulfamethoxazole (Shane et al., 2017). Although resistance to streptomycin (12.2% of isolates tested) was the most prevalent, resistance to all of these recommended treatment options occurred – ampicillin (8.5% of isolates tested), ceftriaxone (6.8% of isolates tested), ciprofloxacin (5.8% of isolates tested), trimethoprim-sulfamethoxazole (2.4% of isolates tested), and azithromycin (0.4% of isolates tested).

Since 97.9% (47/48) of dairy calf-associated salmonellosis tested by AST and/or WGS showed antimicrobial resistance, and since this outbreak has been reopened, this should raise concern about doctors' ability to treat patients given that the past outbreak

strains demonstrated resistance to six to eleven distinct antibiotics from eight of nine drug classes included on the NARMS AST panel. Of specific concern was the high prevalence of resistance to the first line (ciprofloxacin (100%), ceftriaxone (97.9%)) and second-line (ampicillin (97.9%) and trimethoprim-sulfamethoxazole (17%) antimicrobial drugs used to treat severe cases of salmonellosis (Shane et al., 2017). Only azithromycin was not represented among the resistance profiles in this outbreak.

When comparing the epidemiology of patients with antimicrobial resistant strains of *Salmonella* to that of patients with susceptible strains, the median age was lower in the former group for all isolates as well as the subset of live poultry-associated and reptile-associated outbreaks. The higher prevalence of hospitalization for cases with resistant isolates compared to susceptible isolates indicated a greater severity of infection and was consistent with findings in foodborne outbreaks of non-typhoidal *Salmonella* (Brown et al., 2016).

Resistance Concordance

Although at first pass AST and WGS predicted resistance results are concordant in 78% of isolates (90/116), a further 16% of isolates were explained by differences between the standard NARMS AST panel and WGS testing as well as known relationships between resistance genes and phenotypic resistance related to trimethoprim-sulfamethoxazole, chloramphenicol, and nalidixic acid. This left only 6% of isolates whose discordance was theorized to be due to possible plasmid loss in the bacteria between sampling and testing, the known lower specificity and positive predictive value for streptomycin that has since prompted a change in NARMS interpretive criteria for streptomycin (McDermott et al., 2016), and limitations of

interpretations on susceptibility testing (i.e., defining cut-off points at the edges of the susceptibility “bell curve”).

Since most state public health laboratories are certified to do whole genome sequencing rather than having to ship samples to the CDC for testing, it is likely that WGS will become more common in outbreak investigation and response. As noted by McDermott et al., culture-independent methods including whole genome sequencing are becoming more common as they allow a single workflow and do not require labs to maintain “quality typing sera” and specialized training and reagents” (2016). Therefore it is vital that there is an understanding of ways in which AST and Pred-R may not align as well as how to interpret these instances of discordance to help clinicians make clinical choices and to understand outbreak epidemiology. By demonstrating which types of resistance discordance are common amongst zoonotic outbreak, the NARMS team can develop standardized guidance and protocols for testing and interpretation of results that will help health care professionals make decisions about antimicrobial use for patients with animal-associated salmonellosis.

Limitations

Underdiagnosis and underreporting of enteric illness can occur at any point along the surveillance pathway from the individuals who are minimally affected or who cannot or will not seek help from a healthcare provider, to lack of diagnosis or testing by health care provider, or failing to report or submit samples to the relevant public health authority of outbreak reporting system. In these cases, it is possible that underreporting of subclinical or mild cases will bias the case population to more severe disease resulting in hospitalization or death. While some data for patients were close to

complete or complete (e.g., age, gender, exposure to the vehicle), other data was lacking (e.g., race, ethnicity) or variable depending on the outbreak (e.g., hospitalization, death). In the latter case, it is possible that the cases missing data about hospitalization or death might have been different in some way from the cases for whom data were present.

While it might have been interesting to investigate trends in resistance over time for zoonotic outbreaks, there was only one outbreak in guinea pigs and one in dairy calves, and the outbreaks from 2015 and 2016 have much smaller proportions of isolates that had resistance testing than in outbreaks from 2017 to 2018. In the future, more trends may emerge as the proportion of samples tested increases and hqSNP and wgMLST trees continue to be the norm for outbreaks.

Since only 11.3% (116/1030) of those tested had both AST and Pred-R, it was challenging to make conclusions about the nature of resistance concordance between the two testing modalities when it comes to zoonotic salmonellosis outbreaks. A further 17 additional Pred-R results could not be downloaded from the PulseNet computer due to technical issues with the hardware but will be analyzed if become available.

In this study, we did not include plasmid DNA as part of our resistance profile because the short reading sequence does not allow the lab to definitively prove whether the gene is on the plasmid or the bacteria itself. It is expensive and time-consuming to confirm the locations of genes; therefore, only the presence of resistance genes and where relevant, the presence of a partial or full mutation in the quinolone resistance determining region that would predict intermediate or complete resistance to nalidixic acid (i.e., Nal) and ciprofloxacin (Cip), were included in this study.

Opportunities for Further Research

Given the continuing increase in proportion of isolates undergoing WGS, more data will be available to better characterize both the epidemiology and resistance profile of zoonotic outbreaks of salmonellosis. Since WGS is increasingly prevalent as a testing methodology due faster results and increased laboratory certification, it is important to understand whether this newer technology can be as useful as the older AST. AST is necessary as it allows labs to obtain PFGE pattern for outbreak surveillance and response and shows the true behavior of the bacteria and whether the resistance genes it has are expressed in vivo. To further explore resistance concordance and mechanisms of discordance between results, a systematic analysis of isolates from zoonotic outbreaks of *Salmonella* should be undertaken. Given the cost associated with such testing, resources should be directed to outbreaks with that have shown the highest prevalence of discordance (i.e., dairy calves, nine of 26 discordant samples) and antimicrobial resistance. Since this outbreak investigation has been reopened in 2018, this could be done prospectively.

Although WGS trees are typically constructed during active outbreaks and finalized when an outbreak is closed, the CDC performs active surveillance by reaching out to state and large metropolitan public health departments to request representative outbreak samples, and states submit their requisite one of every 20 *Salmonella* samples to PulseNet as part of passive surveillance. This is reflected in the zoonotic outbreaks in this study as only 44% of resistant isolates (n=87) were included on the finalized WGS trees for outbreaks. Since WGS and resistance data for outbreak isolates are often generated after outbreaks are closed, this reduces a bias toward resistant isolates

because resistance patterns for outbreaks are not generally known at the time of outbreak closure. Resource and time permitting, new outbreak WGS trees could be constructed that retrospectively include additional cases that have been sequenced but were not included on the original trees to better understand how WGS tree clade position and/or PFGE pattern relate to resistance patterns. For example, the 2017 outbreaks of *Salmonella* Braenderup, Hadar and Typhimurium linked to live poultry had 43, 35, and 45 isolates, respectively, on the final WGS tree, but there were a further 103, 106 and 98 isolates respectively that have sequencing data that could be incorporated into a new tree. Similarly for the complex and highly resistance isolates from the outbreak of *Salmonella* Heidelberg attributed to dairy calves, there were 15 isolates on the WGS tree but a further 33 with sequencing data. Recreating a WGS tree is not typically done once an outbreak is considered closed; however, in light of the complicated patterns of resistance, it might be possible to request that a new tree be created.

While not analyzed in this study, isolates from food (e.g. chicken, pork, beef), animals (e.g., swabs, organs, and feces), and the environment were present in the majority of outbreaks and found to be highly related by PFGE and WGS. They were present in the outbreak hqSNP or wgMLST trees and have been part of the standard NORS outbreak closeout summaries. Statistical testing including Fisher's exact and Wilcoxon rank sum tests should be used to compare the prevalence of phenotypic and genotypic resistance patterns between non-human and human isolates as well as to further characterize the statistical significance of any differences noted in epidemiologic variables and health outcomes between patients with resistant and susceptible isolates

or between patients from outbreaks of the different vehicle types. These tests will increase the level of understanding of the source and spread of bacterial isolates between hosts directly or through fomites including those with antimicrobial resistance.

Conclusions

Zoonotic outbreaks of *Salmonella* present ongoing challenge to the health of the public and in particular vulnerable groups such as children aged <five years and adults aged >65 years. The continued presence of highly related isolates from year to year indicates a persistent need for outbreak investigation and public heath interventions. Given that the prevalence of hospitalization, a proxy for case severity, was as high as one of every three cases for live poultry, reptile, and dairy calf-associated outbreaks, these outbreaks represent a serious threat to public health. They indicate a need to strengthen and support surveillance systems between municipal, state, and federal partners and will require coordination of experts in human and animal health as well as collaborations with individuals who are empowered to make changes in practices throughout the animal distribution chain from the point of origin, such as a producer or breeder to consumer.

Even as there is a switch from traditional culture and susceptibility testing to culture-independent and genetic testing, reflex cultures for positive samples is required to elicit isolate PFGE patterns and support outbreak surveillance mechanisms through PulseNet and NORS. Further research that includes performing phenotypic and genotypic resistance testing on patient isolates from zoonotic salmonellosis outbreaks will be needed to understand whether interpretation of resistance discordance in foodborne outbreaks of Salmonella can be extrapolated to these outbreaks as well.

Finally given the continued budgetary and personnel pressures that public health departments face, it is vital to understand the most efficient use of public health dollars and to support research into methods that allow more testing for the same amount of money without creating undue burden on state and local health departments.

CHAPTER 4 PUBLIC HEALTH LEADERSHIP CONTEXT OF ZOONOTIC SALMONELLOSIS AND ANTIMICROBIAL RESISTANCE

4.1 Public Health Threat of Zoonotic Salmonellosis and Antimicrobial Resistance

Antimicrobial resistance is a growing concern at the local level for health care providers and hospitals and at the national (e.g., AVMA, CDC, FDA, USDA) and global level where organizations such as the World Health Organization (WHO), World Organisation for Animal Health (OIE), and the Food and Agriculture Organization (FAO) are working independently as well as in a triagency coordination to combat antimicrobial resistance. The CDC has published Core Elements of Antimicrobial Stewardship for Hospitals (CDC, 2014), Nursing Homes (CDC, 2015), and Outpatient Clinics (Sanchez et al., 2017) as well as specific applications for small and critical access hospitals whose needs and resources may differ from their larger counterparts (5CDC, 2017). Similarly in veterinary medicine, individual species groups have adopted judicious use and antimicrobial stewardship guidelines, and the AVMA's Committee on Antimicrobials drafted and the House of Delegates unanimously passed the Definition and Core Elements of Antimicrobial Stewardship in Veterinary Medicine in January of 2018 (AVMA, 2018). This work in human and animal medicine was made possible through the contributions of many stakeholders representing both professional organizations as well as the perspectives of the frontline health care providers to apply the science of what is known about antimicrobial stewardship to its practical applications.

Although more research into the mechanisms by which antimicrobial resistant strains of bacteria spread is needed, the trans global movement of people, animals, and goods has been implicated in some outbreaks since related resistant strains of bacterial isolates have been found across the globe in humans, animals, and the environment. Of the estimated 1.2 million cases of non-typhoidal *Salmonella* in the US annually, the CDC reports that 1.1 million are acquired domestically, which translates into the remaining 100,000 people (8.3%) with internationally-acquired infections (CDC, *Questions and Answers* | *Salmonella*, 2018,). In addition, the 9% of domestically acquired infections were thought to be acquired through non-food sources such as contaminated pet food, infected animals, and the environment where animals are housed (e.g., aquariums, pens, cages)

While this analysis focused on the epidemiology and resistance patterns of human patients linked to multistate outbreaks, isolates from food (e.g. chicken, pork, beef), animals (e.g., swabs, internal organs, cecal samples, or fecal samples), and the environment (e.g., turtle water or aquarium swab, duck straw, bedding, enclosures) were analyzed during these outbreaks and been found to be highly related through WGS. Since these zoonotic outbreaks typically covered a large number of states, the need for and strength of the NNDSS, NORS and PulseNet for passive and active surveillance is evident. It also requires the active engagement of physicians, veterinarians, laboratorians, public health professionals and federal partners in the FDA and the Food Safety and Inspection Service (FSIS) within the Animal, Plant Health Inspection Service (USDA APHIS).

In addition to finding closely related isolates from multiple states or from multiple sources (human, animal, food, environment), PFGE or WGS matches in PulseNet from past outbreaks suggest that bacterial strains can persist and spread. For example, the 2017 outbreaks of *Salmonella* Agbeni linked to turtles contained patient isolates that were closely related to a 2016 outbreak and samples from a street vendor selling turtles in 2015 (CDC, Multistate Outbreak of *Salmonella* Agbeni Infections Linked to Pet Turtles, 2017 (Final Update), 2018). Similarly outbreaks of *Salmonella* related to live poultry, of which there were 53 between 1990–2014 (average 3.5 outbreaks/year) and 23 between 2015–2018 (average 7.7 outbreaks/year), show related samples from past years (e.g., *Salmonella* Hadar and Muenster outbreaks associated with live poultry that where 2015 outbreak isolates were highly related to 2016 isolates) (Basler et al., 2016; CDC, *Don't Play Chicken With Your Health*, 2018).

Finding a historical isolate as part of an ongoing outbreak can also provide insight into whether control measures or interventions that had been suggested were implemented by the source of the outbreak vehicle. A good example of the consequences of failing to implement public health interventions is underscored by the outbreak of salmonellosis related to guinea pigs, which was considered closed in 2018. Patients in this outbreak were exposed between July 2015 and December 2017; however, a patient sample from 2010 was found to only one SNP different than a patient in this later outbreak (Robertson et al., 2018). In this case, a genetic link between isolates revealed a common wholesaler at the beginning of the supply chain that led to individuals purchasing guinea pigs, and it was found that prior interventions including environmental testing were not implemented (Robertson et al., 2018).

4.2 One Health and Antimicrobial Stewardship

Given the ongoing and persistent outbreaks of zoonotic salmonellosis that span human, animal, and environmental domains, addressing antimicrobial resistance will not be easily tackled by a single entity or domain. As defined by the AVMA, antimicrobial stewardship for veterinary medicine is “the actions veterinarians take individually and as a profession to preserve the effectiveness and availability of antimicrobial drugs through conscientious oversight and responsible medical decision-making while safeguarding animal, public, and environmental health.” The goal to preserve the effectiveness and availability of antimicrobials drugs is shared by human, animal and public health counterparts; however, the capability of resistant bacterial populations to spread from person to person, from the environment (e.g., soil, water and air), and to and from animals, means that collaboration solely between health care sectors will not be adequate to address this problem. A problem this widespread, serious and complex demands systems-level thinking and the engagement of stakeholders in agriculture, policy making, industry groups and businesses focused on animal breeding, distribution, and sale, and pharmaceutical groups and manufacturers researching and developing new antimicrobials, antimicrobial alternatives, and preventive care (e.g., vaccines, immune modulators) in a One Health approach.

4.3 Public Health Professionals as Chief Health Strategist

My work on the AVMA Committee on Antimicrobials and my practicum with colleagues at the CDC including those in ORPB and NARMS have expanded my understanding of the complexity of the issue of zoonotic disease and antimicrobial resistance and the relevance of the concept of the public health professional as the

Chief Health Strategist proposed by the DeSalvo et al. (2016). Rather than doing all of the work, the chief health strategist must engage and mobilize community partners to act to improve the “upstream” social determinants of health (DeSalvo et al., 2016; RESOLVE, 2014). A successful public health leader should adopt the principles of transformational leadership in which the leader creates a collective vision that inspires others to take action. After exploring the public health threat of zoonotic salmonellosis and antimicrobial resistance from the perspectives of infectious disease, surveillance, policy, global health, research and leadership perspectives, I recognize that a common theme to taking next steps in all of these areas is identifying and engaging stakeholders.

4.4 Stakeholder Analysis

No matter the intervention, it is critical to define not only who the stakeholders are but also to determine what their current level of support and influence is, how influential their participation will be in the change process, and how and when to engage them in interventions. An effective public health leader should be able to help stakeholders understand their role in the complex web of an issue such as antimicrobial resistance and help them appreciate how their involvement is critical to the success of the intervention. It is also helpful to repeat the stakeholder analysis using different tools depending on what action you might take while taking care to consider stakeholders at all levels of the socioecological model (individual, interpersonal, community/organizational, and societal/policy). There are multiple ways to analyze the stakeholders in an issue, and I have included sample figures that I developed to consider stakeholders for live poultry-associated salmonellosis (Figures 4.1 – 4.4).

To begin, a STEP scan is useful for brainstorming what individuals or groups are affected or influential and assigning them to the relevant Social, Technical, Economic, and Political groups (Aguilar, 1967). In the case of live poultry this might include the distribution network from breeders to distributors to stores and finally customers (Figure 4.1). With small adjustments, this and the other stakeholder figures could apply to the other animal vehicles covered in this study (reptiles, dairy calves, or guinea pigs).

Secondly the Center for Community Health and Development's Community Tool Box takes the perspective of primary, secondary and tertiary stakeholders (Community Tool Box, 2017). Their designations are tiered based on who is affected (primary), who is responsible for those affected (secondary), and who can exert influence to affect change for the public health threat. The type of influence an individual or group has can then relate back to the original STEP scan as social, technical, economic and policy influence are all involved particularly in a complex issue such as antimicrobial resistance.

Next, I would consider each stakeholder in their level of current support as well as the level of support needed to promote health behavior changes that support infection control and antimicrobial stewardship along the lines of the International Association for Public Participations (IAP2) Spectrum of Public Participation and Type of Involvement from Stakeholders (www.iap2.org). It is important to perform this analysis to gauge how to efficiently use limited public health resources (i.e., money, people, supplies). In the case of live poultry-associated salmonellosis veterinarians, public health departments, and pediatricians could be expected to support for interventions to protect the health of children; however, pediatricians are more likely than either of the

other groups to influence others to make a change since they are trusted figures in the lives of parents of young children and are authority figures due to their medical degrees. (Figure 4.2). By contrast, hatcheries and feed store owners may resist public health interventions seen as intrusive and burdensome to their business practice; however, despite this expected resistance, if they can support change, their behavior is expected to have a moderate effect on improving public health.

Once the decision has been made to engage particular stakeholders, the IAP2 Spectrum of Public Participation is helpful in deciding whether it is most appropriate to inform, consult, involve, collaborate or empower each stakeholder. Each level allows more participation by the stakeholder and shifts the responsibility for making and implementing decisions from a more centralized to a more diffuse structure (Figure 4.3) (IAP2, 2014). On the low end of participation, the authority figure or institution only interacts with the public to educate and inform them about the problem or what behaviors to adopt to protect themselves. On the upper end of participation, the public is empowered to make the decisions, and the public health organization commits to implementing what the public decides. In my example of live poultry-associated salmonellosis, I would chose to seek collaborations with day care and preschool educators. These educators are secondary stakeholders since they care for children aged <5 years, who are overrepresented in *Salmonella* outbreaks, and their influence is high because they decide whether baby chicks or ducks will be handled in the classroom (CDC, *Salmonella infection | Healthy Pets, Healthy People*, 2018). In that case, it was important to assimilate the educator expertise into the context of *Salmonella* prevention to collectively determine what options the educators preferred

and would be able to implement (IAP2, 2014). In contrast, subject matter experts on salmonellosis and poultry (e.g., CDC, veterinarians) would be consulted to inform the issue but their involvement in decision making would be less because they don't set protocols or implement preventive strategies in schools or daycares.

4.5 Leadership at all levels

The result of all these ways of looking at stakeholders in the context of zoonotic salmonellosis and antimicrobial resistance results is a mental model and stakeholder map that is a complex web of interactions (Figure 4.4). To build the trust and vision that change is both necessary and possible requires a transformational leader with keen emotional intelligence to understand what is important for themselves and others and who can use persuasive tools to convince others of the vision and its possibility, express confidence in themselves and others, and demonstrate how it can be done (Yukl, 2012, pg. 332-5). The benefits of adopting a transformational leadership style were demonstrated by Carlton et al. (2015), who found strong and statistically significant correlations between a leader's transformational leadership style and leadership outcomes (e.g., extra effort, effectiveness and satisfaction), which did not hold for leaders who self-described as transactional leaders (i.e., ones who used contingent awards or active management-by-exception).

While Yukl describes transformational leadership as a group of behaviors (idealized influence, individual consideration, inspirational motivation, and intellectual stimulation), a survey of public health professionals in the UK found that public health superheroes were commonly found to have talents in mentoring-nurturing, shaping-organizing, networking-connecting, knowing-interpreting and advocating-impacting

(Yukl, 2012, pg. 322; Day et al., 2014). Kouzes and Posner go further and describe 5 leadership behaviors that put the theory of transformational leadership into practice – Model the Way, Inspire a Shared Vision, Challenge the Process, Enable Others to Act, Encourage the Heart (2012). The promising conclusion is that these effective leadership traits and skills can be encouraged and taught to anyone in a process of lifelong learning not just a select few who have them innately (Day et al., 2014; Kouzes and Posner, 2012; Yukl, 2012). Using the added step of creating organizational or inter-organizational support systems as proposed by Upshaw (Module 2, Lesson 6, 9/25/2017) aids the process of continual improvement in transformational leadership. By taking a perspective of promoting 'leadership at all levels,' and considering that "leadership is everyone's business' a transformational leader can enhance the process of making connections and building up support systems by empowering others to lead (Day et al., 2014; Kouzes and Posner, 2012).

Leadership is required at every level of the society from individuals and organizations (e.g., schools, hospitals, feed stores) to policy makers and should focus on stakeholders identified as playing an important role in contributing to the success of the intervention. When thinking outside the more natural realm of health whether animal or human, the importance of leadership from other groups becomes apparent. When applied to zoonotic salmonellosis, and in particular, outbreaks linked to live poultry, some stakeholders whose leadership is essential to carrying the message and practice of prevention are:

1. Breeders and suppliers – Helping businesses take ownership of their role in rearing and raising healthy animals, implementing biosecurity and infection

control protocols to keep facilities clean and free of disease while acknowledging that these actions decrease but do not eliminate the possibility of *Salmonella* carriage in live birds

2. Distributors – Emphasizing infection control and improved signage on shipping containers with live birds
3. Feed and farm stores - Encouraging feed store owners that sell live poultry to take action to limit customer access to birds, particularly children, through use of barriers and fencing around displays of chicks and to prevent young children handling chicks
4. Pet stores – Aiding management in crafting good sanitation, hygiene and antimicrobial stewardship protocols and in ensuring compliance by local stores and employees

4.5 Change Management

As a public health leader it will be critical to help these and other organizations see their role in the complex stakeholder map, to persuade these groups that zoonotic infections and antimicrobial resistance is something they should care about, and to move these critical organizations and individuals through a change process that improves infection control, reduces the inappropriate use of antimicrobials and supports rapid outbreak identification and response both on the human and animal sides while leaving in place support structures that makes the change sustainable. Yukl describes the 4 stages of change that individuals and organization must go through when confronted with a request to change. These are overcoming denial, channeling anger, mourning without depression and optimism about adaptation (Yukl, 2012, pg. 79-81). In

order to get buy-in from stakeholders, barriers to change will need to be overcome including perceptions that change is not necessary, not feasible, not cost-effective or that there is a lack of established trust between stakeholders.

While it is beyond the scope of this paper to go into change management strategies in details, the need for public health leaders to continually improve their skills in mentoring-nurturing, shaping-organizing, networking-connecting, knowing-interpreting and advocating-impacting holds true despite variations in the type and complexity of barriers to change (Day et al., 2014). These skills will be needed to address stakeholders both within and outside the health care because the existing mental model for many is to view antimicrobial drugs as plentiful and essential for treating symptoms of illness. For those with this mental models, there is no consideration as to how to use antimicrobials more judiciously because there is no awareness that antimicrobials are not appropriate in every case of sneezes and sniffles, for example, that resistant bacterial strains are becoming more prevalent making many drugs useless where previously they were effective, and that treating resistant infections comes at a medical, economic, and physical cost to the patient and health care system. An increased awareness, a culture shift, and sometimes difficult behavior changes will be required on the part of patients and prescribers (both human and veterinary) to reduce the demand for and the inappropriate prescribing of antimicrobials.

4.6 Conclusion

Combatting antimicrobial resistance and preserving the effectiveness of antimicrobial drugs will require collaboration and synergy at all levels for progress to be made. Leaders with an understanding of the urgency of the threat to health and a vision

of what is possible will be needed at all levels of the socioecological model as well as from all four quadrants of the STEP scan. A perspective of continuous learning and an optimism that leadership skills can be learned and improved as well as a commitment to sharing these skills and knowledge with others to increase their leadership potential is key to inspiring and beginning the change process as well as to creating strong support structures that will sustain changes that preserve the effectiveness and availability of antimicrobial drugs and promote the promote the health and well being of people and animals.

Table 1: Antimicrobial agents used for susceptibility testing for Salmonella isolates by the NARMS laboratory in the US, 2015–2018

CLSI Class	Antimicrobial Agent	NARMS Code	Antibiotic Short Key
Aminoglycosides	Gentamicin	Gen	GEN
	Streptomycin	S	STR
Beta-lactams/Beta-lactamase inhibitor combinations	Amoxicillin-clavulanic acid	Au	AUG
Cephems	Ceftriaxone	Cx	AXO
	Cefoxitin	Fox	FOX
Folate pathway inhibitors	Sulfisoxazole	Su	SMX
	Trimethoprim-sulfamethoxazole	Cot	COT
Macrolides	Azithromycin	Azm	AZM
Penicillin	Ampicillin	A	AMP
Phenicols	Chloramphenicol	C	CHL
Quinolones	Ciprofloxacin	Cip	CIP
	Nalidixic acid	Nal	NAL
Tetracyclines	Tetracycline	T	TET

Source: https://www.cdc.gov/narms/reports/annual-human-isolates-report-2015.html (Table 2)
https://www.cdc.gov/narms/antibiotics-tested.html#modalIdString_CDCTable_0 (Table Salmonella)

Table 2: Comparison of case and outbreak counts based on proportion and number of isolates with whole genome sequencing in zoonotic salmonellosis outbreaks in the US, 2015–2018

	Live Poultry			Reptiles			Dairy Calves			Guinea Pigs		
	All	**>25%**	**>25%,≥ 10**	**All**	**>25%**	**>25%,≥ 10**	**All**	**>25%**	**>25%,≥ 10**	**All**	**>25%**	**>25%,≥ 10**
Outbreaks	23	10	9	7	6	5	1	1	1	1	1	1
Cases	2287	1121	1110	338	236	214	56	56	56	10	10	10
% of total cases	85.0%	78.8%	79.9%	12.6%	16.6%	15.4%	2.1%	3.9%	4.0%	0.4%	0.7%	0.7%
Cases with AST and/or WGS	855	713	706	117	117	110	48	48	48	10	10	10
% of total tested	83.0%	80.3%	80.8%	11.4%	13.2%	12.6%	4.7%	5.4%	5.5%	1.0%	1.1%	1.1%
% tested by vehicle	37.4%	63.6%	63.6%	34.6%	49.6%	51.4%	85.7%	85.7%	85.7%	100%	100%	100%

>25% = Subset of outbreaks with at least 25% of case isolates having whole genome sequencing
>25%, ≥10 = Subset of outbreaks with at least 25% of case isolates having whole genome sequencing totaling 10 or more isolates

Table 3: Comparative Epidemiology of Outbreaks of Zoonotic Salmonellosis in the United States between all outbreaks, a subset of outbreaks that had WGS for > 25%, and a subset of outbreaks with at least 10 isolates with WGS that constitute > 25% of isolates, 20

	All outbreaks		>25% Sequenced		>25% & ≥10 Sequenced	
Outbreaks (#)	**32**		**18**		**16**	
Cases (#)	**2691**		**1423**		**1390**	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**
Source						
Blood	0.0%	22.4%	0.0%	22.4%	0.0%	22.4%
Stool	64.5%	100.0%	64.5%	100.0%	64.5%	100.0%
Urine	0.0%	16.7%	0.0%	16.7%	0.0%	16.7%
Other	0.0%	9.1%	0.0%	9.1%	0.0%	9.1%
Age (years)						
Minimum	0	1	0	1	0	1
Maximum	53	106	53	**101**	53	**101**
Median	1	56	1	56	1	56
Gender						
Female (%)	41.5%	81.8%	41.5%	81.8%	41.5%	81.8%
Race						
White (%)	35.7%	100%	35.7%	100%	35.7%	100.0%
Black (%)	0%	50.0%	0%	50.0%	0.0%	50.0%
Other (%)	0%	**18.9%**	0%	**16.7%**	0.0%	**16.7%**
Ethnicity						
Hispanic/Latino (%)	0%	68.8%	0.0%	68.8%	0.0%	68.8%
Hospitalized						
Yes (%)	12.5%	55.6%	12.5%	55.6%	12.5%	**47.6%**
Died						
Yes (%)	0%	9.1%	0%	9.1%	0.0%	9.1%
Exposure to vehicle						
Yes (%)	9.5%	**100.0%**	9.5%	**88.9%**	9.5%	**88.9%**
Data Availability						
Age	91.7%	100.0%	91.7%	100.0%	91.7%	100.0%
Gender	90.0%	100.0%	90.0%	100.0%	90.0%	100.0%
Race	0.0%	70.0%	0.0%	70.0%	0.0%	70.0%
Ethnicity	0.0%	70.0%	0.0%	70.0%	0.0%	70.0%
Hospitalization	**56.5%**	100%	**61.1%**	100%	**61.1%**	100.0%
Death	**56.5%**	100%	**61.1%**	100%	**61.1%**	100.0%
Exposure	49.4%	**100.0%**	49.4%	**94.7%**	49.4%	**94.7%**

* Bold format indicates difference from other subsets of outbreaks
>25% = Subset of outbreaks with at least 25% of case isolates having whole genome sequencing
>25%, ≥10 = Subset of outbreaks with at least 25% of case isolates having whole genome sequencing totaling 10 or more isolates with whole genome sequencing
Demographic proportions are a percent of isolates for which results were recorded. Demographic questions left blank or marked as 'unknown,' 'don't know,' or 'did not answer' were excluded.
Age of 0.00 refers to age less than 1 month.

Table 4: Human Cases of Zoonotic Salmonellosis Compared by Implicated Vehicle and Presence of Antimicrobial Resistance in the US, 2015–2018

	Live Poultry				Reptiles				Dairy Calves		Guinea Pigs	
	All	AMR	No AMR	NR	All	AMR	No AMR	NR	All	AMR	All	AMR
Outbreaks	23	11	7	5	7	3	3	1	1	1	1	1
Cases												
#	2287	1522	529	236	338	166	70	102	56	56	10	10
%	85.0%	86.8%	88.3%	69.8%	12.6%	9.5%	11.7%	30.2%	2.1%	3.2%	0.4%	0.6%
Isolates with AST or PredR												
%	37.0%	50.1%	16.1%	N/A	34.6%	50.0%	48.6%	N/A	85.7%	85.7%	100%	100%
#	847	762	85	N/A	117	83	34	N/A	48	48	10	10
Isolates with AMR												
% of tested	16.8%	18.6%	0%	N/A	6.8%	9.6%	0%	N/A	97.9%	97.9%	10.0%	10.0%
#	142	142	0	N/A	8	8	0	N/A	47	47	1	1
Age (years)*^												
Min	0.00	0.08	0.00	0.00	0.00	0.06	0.00	0.02	0.17	0.17	1.00	1.00
Max	106	106	92	89	100	100	58	83	89	89	70	70
Gender*												
Female (%)	53.3%	54.5%	50.4%	51.9%	54.2%	57.3%	51.5%	51.0%	56.4%	56.4%	55.6%	55.6%
Female (#)	1194	812	262	120	176	90	35	51	31	31	5	5
Hospitalized*												
Yes (%)	28.9%	28.6%	26.3%	38.6%	37.9%	40.7%	40.0%	32.5%	34.7%	34.7%	12.5%	12.5%
Yes (#)	524	345	118	61	96	57	12	27	17	17	1	1
Died*												
Yes (%)	0.3%	0.3%	0.2%	0%	0.4%	0.7%	0%	0%	0%	0%	0%	0%
Yes (#)	5	4	1	0	1	1	0	0	0	0	0	0
Exposure to vehicle*												
Yes (%)	65.7%	54.8%	89.9%	69.5%	51.8%	51.6%	47.2%	54.2%	63.0%	63.0%	75.0%	75.0%
Yes (#)	1118	594	419	105	128	66	17	45	34	34	6	6

*Count and percent of isolates for which results were recorded. Demographic questions left blank or marked as 'unknown,' 'don't know,' or 'did not answer' were excluded.
^ Age 0.0 = infant less than 1 month old
AMR = Antimicrobial Resistance
AST = Antimicrobial Susceptibility Testing
PredR = Predicted Resistance from whole genome sequencing

Table 5: Antimicrobial Resistance in Zoonotic Salmonella enterica outbreaks for antimicrobial drugs included in the standard NARMS AST Panel, 2015–2018

CLSI Class	Antimicrobial Agent	Outbreaks with ≥ 1 resistant isolate)	Outbreaks with resistance (n=26)
Aminoglycosides	Gentamicin	8	31%
	Streptomycin	11	42%
Beta-lactams/Beta-lactamase inhibitor combinations	Amoxicillin-clavulanic acid	9	35%
Cephems	Ceftriaxone	8	31%
	Cefoxitin	7	27%
Folate pathway inhibitors	Sulfisoxazole	10	38%
	Trimethoprim-sulfamethoxazole	6	23%
Macrolides	Azithromycin	4	15%
Penicillin	Ampicillin	9	35%
Phenicols	Chloramphenicol	7	27%
Quinolones	Ciprofloxacin	7	27%
	Nalidixic acid	7	27%
Tetracyclines	Tetracycline	10	38%

Of the 32 possible outbreaks, 26 had antimicrobial susceptibility testing data.

Table 6: Resistance Patterns among Human Isolates of Zoonotic Salmonellosis in the US by Vehicle, 2015–2018 (n=198 isolates with resistance)

Live Poultry	
Pattern	**Resistant Isolates (#)**
A	6
ACSSu	1
ACSSuT	2
ACSSuTCotNalCip(I)GenAzm	1
ACSTAuAzm	1
ASTAuCxFox	1
ASuCot	5
AT	1
AAuCxFox	21
ACip(I)	1
CS	1
CSSu	3
CSSuT	1
CSSuTCot	4
CSSuCot	2
CSSuCotNalCip(I)	1
CSuTCot	1
S	6
SSuTCot	1
SSuTGen	17
SSuGen	27
SSuGenKanAzm	1
ST	8
Su	1
SuCot	1
T	23
NalCip(I)*	3
Cip(I)	1

Reptiles	
Pattern	**Resistant Isolates (#)**
AAuCxFox	2
CTNalCipAzm	1
CNalCip	1
NalCip and NalCip(I)*	4

Dairy Calves	
ACSSuTAuCxFoxCotNalCip	3
ACSSuTAuCxFoxCip(I)	5
ACSSuTAuCxFoxCip(I)Kan	30
ACSSuTAuCxFoxCip(I)KanFos	1
ASSuTAuCxFoxCotNalCip	3
ASSuTAuCxFoxCotCip	2
ASSuTAuCxFoxCip	1
ASSuTAuCxFoxCip(I)KanFos	1
CSSuTCip(I)Kan	1

Guinea Pigs	
Pattern	**Resistant Isolates (#)**
SSuCot	1

Key

NARMS Code	Antibiotic
A	Ampicillin
C	Chloramphenicol
S	Streptomycin
Su	Sulfisoxazole
T	Tetracycline
Au	Amoxicillin-clavulanic acid
Cx	Ceftriaxone
Fox	Cefoxitin
Cot	Trimethoprim-sulfamethoxazole
Nal	Nalidixic Acid
Cip	Ciprofloxacin
Gen	Gentamicin
Azm	Azithromycin

* Resistance pattern seen in more than one type of vehicle.
(I) = Intermediate Resistance

Table 7: Epidemiology of Isolates tested with AST or Pred-R from Zoonotic Outbreaks of Salmonellosis Compared by the Presence Antimicrobial Resistance in the US, 2015–2018

	Resistance (n=198)		No Resistance (n=832)	
	%	(#)	%	(#)
Source Site				
Blood	2.6%	(5)	5.0%	(41)
Stool	89.5%	(170)	87.6%	(715)
Urine	7.4%	(14)	6.3%	(51)
Other	0.5%	(1)	1.1%	(9)
Age (years)				
Minimum	0.08		0.08	
Maximum	89		101	
Median	20		25	
Gender				
Female (%)	52.9%	(100)	55.7%	(451)
Race				
White (%)	100%	(6)	81.8%	(27)
Black (%)	0.0%		15.2%	(5)
Other (%)	0.0%		3.0%	(1)
Ethnicity				
Hispanic/Latino (%)	66.7%	(2)	25.0%	(10)
Hospitalized				
Yes (%)	32.2%	(55)	27.6%	(185)
Died				
Yes (%)	0.0%		0.3%	(2)
Exposure to vehicle				
Yes (%)	68.5%	(111)	56.0%	(339)

Count and percent are for isolates with recorded results. Demographic questions left blank or marked as 'unknown,' 'don't know,' or 'did not answer' were excluded.

Table 8: Epidemiology of Isolates tested with AST or Pred-R from Zoonotic Outbreaks of Salmonellosis Compared by the Presence of Absence of Antimicrobial Resistance by Vehicle, 2015–2018

	Poultry		Reptiles		Dairy Calves		Guinea Pigs	
	AMR (n=142)	No AMR (n=713)	AMR (n=8)	No AMR (n=109)	AMR (n=47)	No AMR (n=1)	AMR (n=1)	No AMR (n=9)
Source Site								
Blood	0.0%	3.6% (25)	0.0%	15.0% (16)	10.6% (5)	0%	0.0%	0.0%
Stool	90.3% (121)	90.0% (629)	87.5% (7)	72.0% (77)	87.2% (41)	100% (1)	100% (1)	88.9% (8)
Urine	9.0% (12)	5.7% (40)	12.5% (1)	9.3% (10)	2.1% (1)	0%	0.0%	11.1% (1)
Other	0.7% (1)	0.7% (5)	0.0%	3.7% (4)	0.0%	0%	0.0%	0.0%
Age (years)								
Minimum	0.08	0.08	0.17	0.17	0.17	N/A	N/A	1.0
Maximum	83.0	101.0	40.0	100.0	89.00	N/A	N/A	70.0
Median	26.0	28.0	9.0	13.0	16.50	N/A	N/A	12.0
Gender								
Female (%)	49.6% (66)	55.4% (385)	87.5%	57.5% (61)	55.3% (26)	100%	100% (1)	50.0% (4)
Race								
White (%)	N/A	100% (6)	N/A	71.4% (15)	100% (5)	N/A	100% (1)	100% (6)
Black (%)	N/A	0.0%	N/A	23.8% (5)	0.0%	N/A	0.0%	0.0%
Other (%)	N/A	0.0%	N/A	4.8% (1)	0.0%	N/A	0.0%	0.0%
Ethnicity								
Hispanic/Latino (%)	N/A	16.7% (1)	N/A	32.1% (9)	100% (2)	N/A	0.0%	0.0%
Hospitalized								
Yes (%)	33.3% (40)	25.2% (145)	0.0%	46.4% (39)	32.6% (14)	N/A	100% (1)	0.0%
Died								
Yes (%)	0.0%	0.2% (1)	0.0%	1.2% (1)	0.0%	N/A	0.0%	0.0%
Exposure to vehicle								
Yes (%)	69.4% (77)	56.3% (296)	80.0% (4)	52.1% (37)	64.4% (29)	100%	100% (1)	71.4% (5)

Count and percent are for isolates with recorded results.
AMR = Antimicrobial Resistance
No AMR = Susceptible to all antimicrobials tested.

Figure 3.1 Relative Prevalence of Implicated Vehicles in Zoonotic Salmonellosis Outbreaks Categorized by the Presence of Outbreak Level Antimicrobial Resistance in the US, 2015–2018

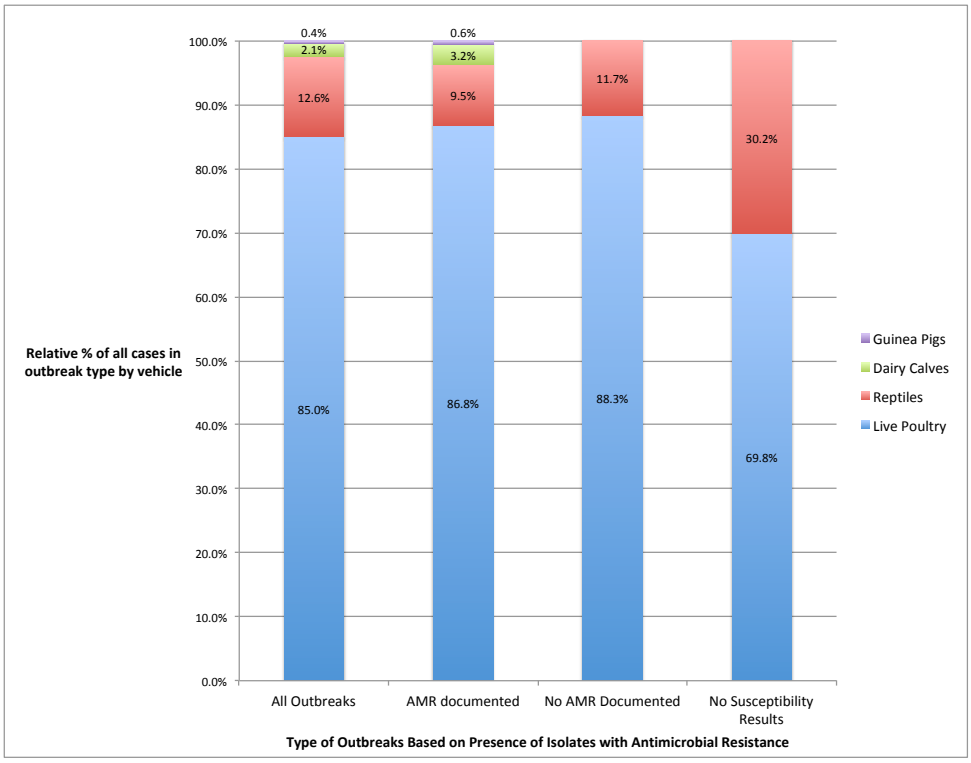


Figure 3.2 Prevalence of Resistance to Individual NARMS Antimicrobial Drugs Among 198 Human Isolates of Zoonotic Salmonellosis with Documented Resistance by AST and/or WGS, 2015–2018

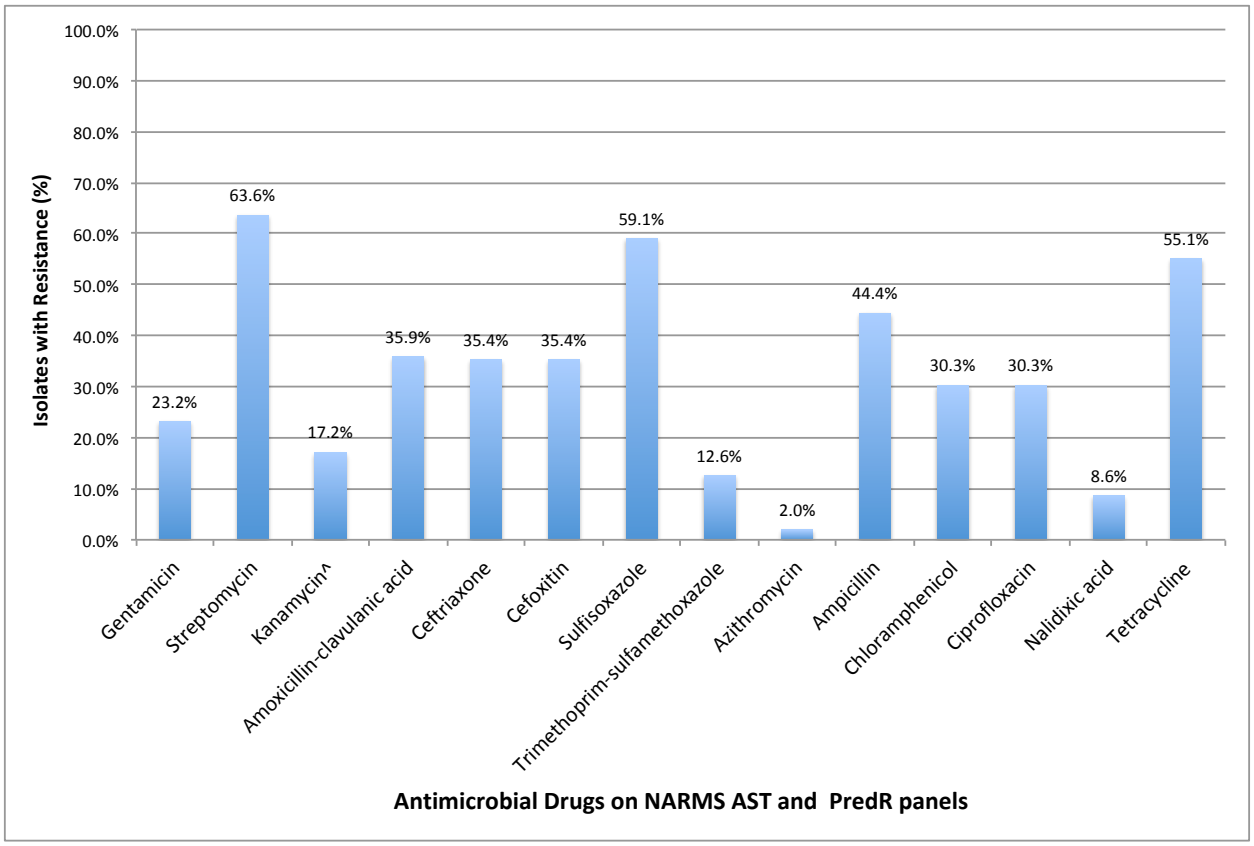
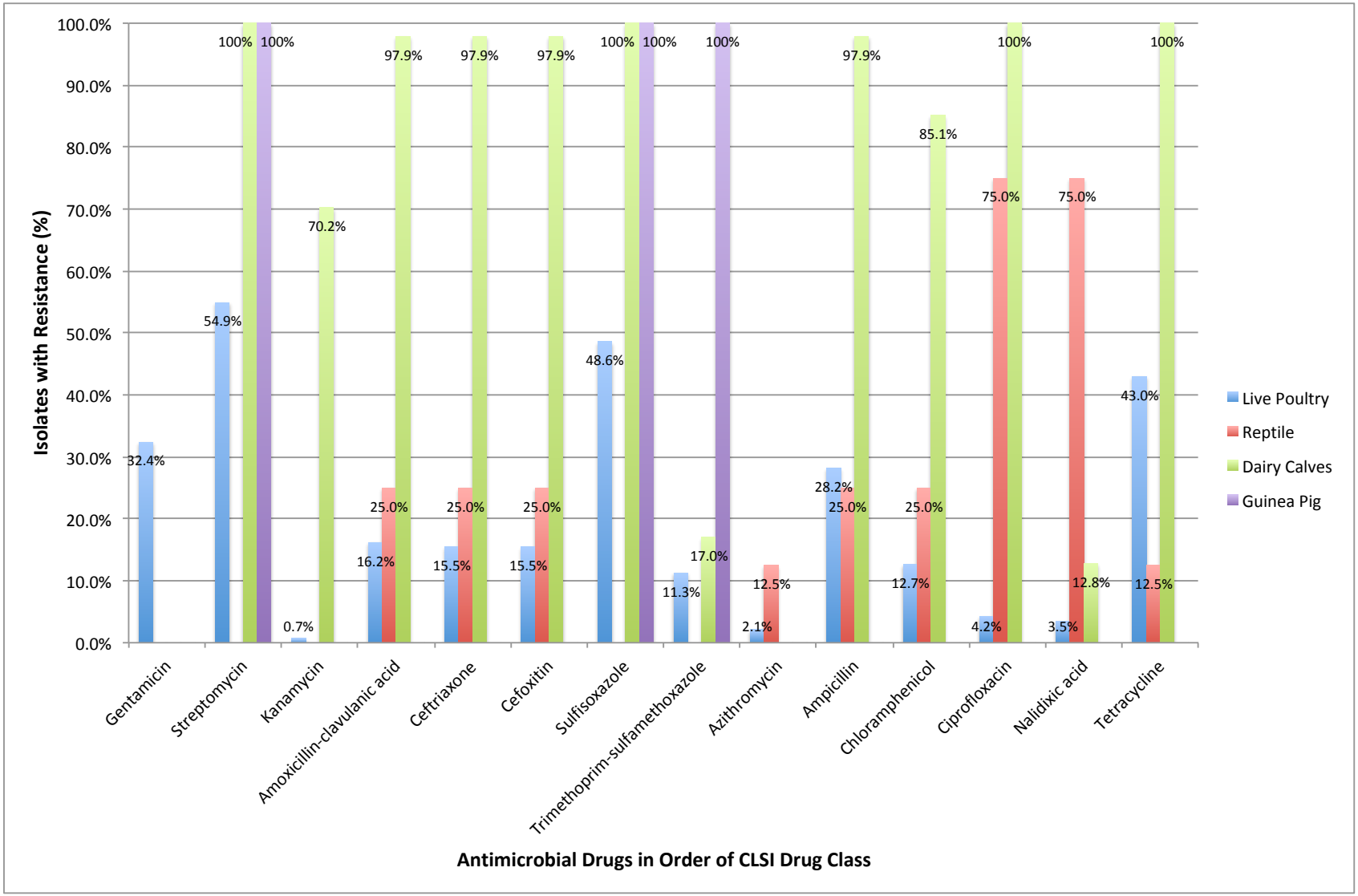


Figure 3.3 Prevalence of Antimicrobial Resistance in 198 Human Isolates of Zoonotic Salmonellosis with Documented Resistance in the US by Vehicle, 2015–2018



* Kanamycin is not on the standard NARMS AST panel but is part of genetic resistance testing.

Figure 4.1 Example of STEP Scan for Stakeholders in the Context of Live Poultry Associated Salmonellosis in the United States

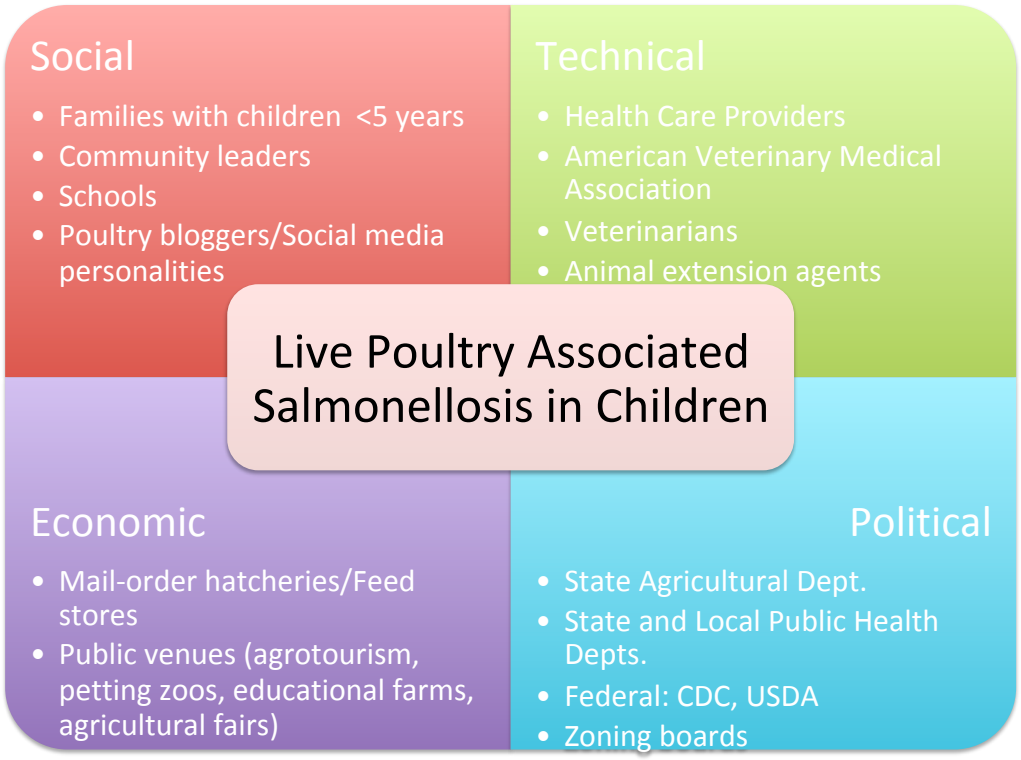
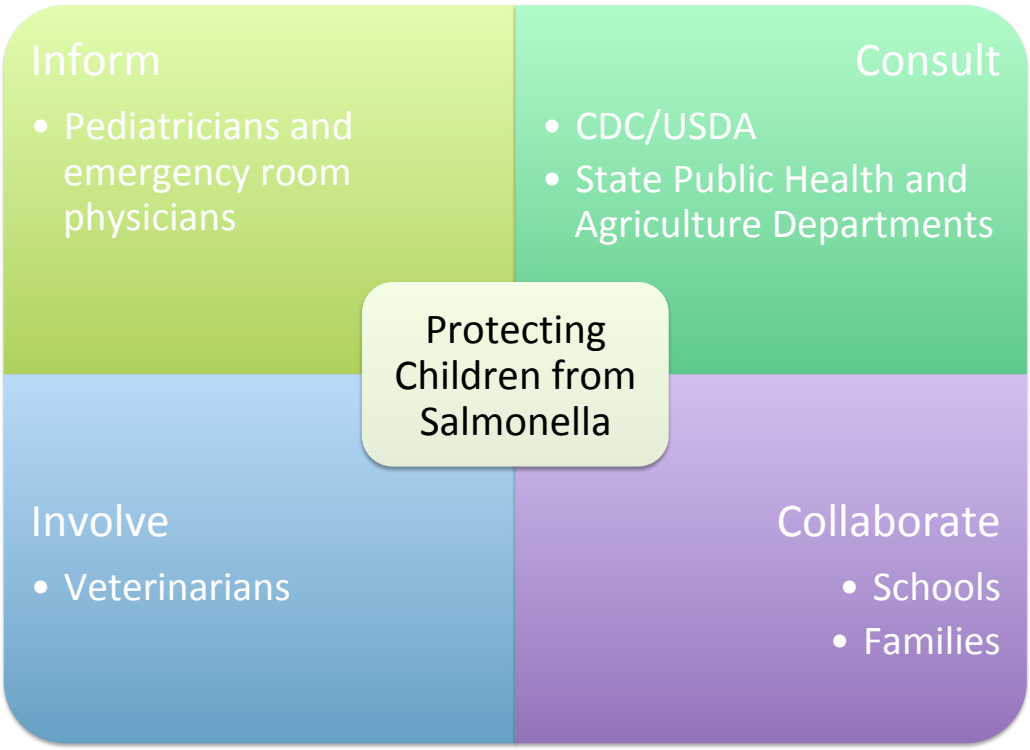


Figure 4.2 Example of the IAP2 Spectrum of Public Participation in the Context of Live Poultry Associated Salmonellosis in the United States

Contribution to Success				
Current Status		Low	Medium	High
	Support	AVMA	CDC Public health dept. Veterinarians	Pediatricians
	Neutral	Zoning boards Extension agents USDA State Ag. Dept.	Community leaders Poultry bloggers (social media)	Families Schools
	Resist		Public venues/ agrotourism Hatcheries Feed stores	

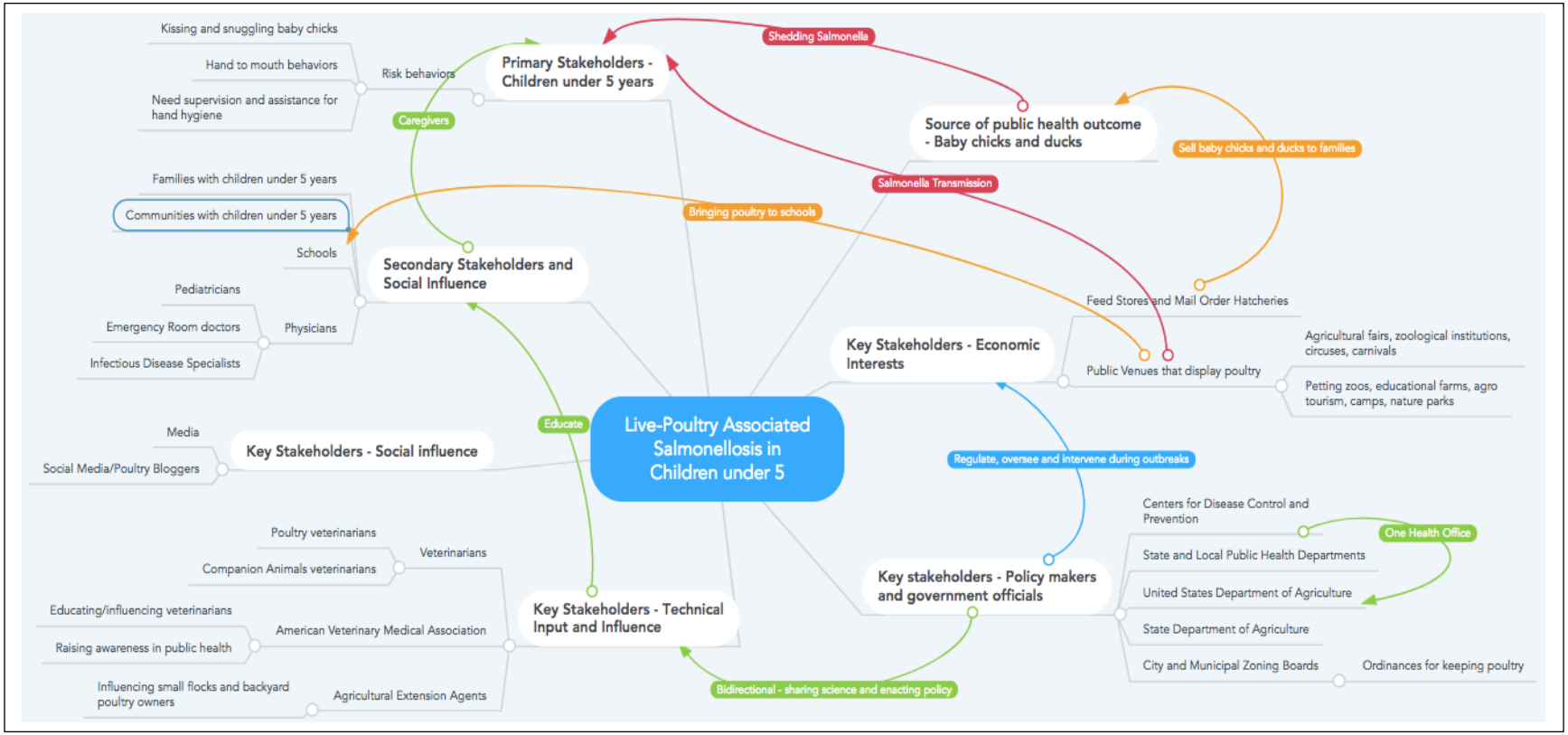
Source:https://cdn.ymaws.com/www.iap2.org/resource/resmgr/foundations_course/IAP2_P2_Spectrum_FINAL.pdf

Figure 4.3 Example of IAP2's Type of Involvement in the Context of Live Poultry Associated Salmonellosis in the United States



Source: www.iap2.org

Figure 4.4 Example of Stakeholder Mapping in the Context of Live Poultry Associated Salmonellosis in the United States



APPENDIX 1. RESISTANCE PROFILES FOR OUTBREAKS OF ZOONOTIC SALMONELLOSIS BY WHOLE GENOME SEQUENCING TREE CLADE DESCRIPTIONS, 2015-2018

Outbreak	WGS Tree Clade	Human Isolates	Distance within Clade	PFGE Pattern	Resistance Pattern	Isolates with AMR (#)
1605MLJBP-1 Braenderup	A (Main)	25	0-14	JBPX01.0039	AAuCxFox	4
Live Poultry			29-75 to B	JBPX01.0039	S	2
				JBPX01.0039	SSuGen	2
				JBPX01.0039	SSuGenKanAzm	1
	B	5		JBPX01.0039	None	0
1604MLTDK-1 Hadar	A	17	0-14	TDKX01.0049	T	1
Live Poultry						
1604MLJFX-1 Infantis	A (Main)	19	0-16	JFXX01.0081	T	5
Live Poultry			55-72 to next clade	JFXX01.0081	SSuTGen	1
	B	7	0-6	JFXX01.0081	T	1
	C	1	17-25	JFXX01.0081	None	0
1704MLTDK-1 Braenderup	A	27	0-7	JBPX01.0002	A	1
Live Poultry			124-213 to Clade B	JBPX01.0002	AAuCxFox	2
				JBPX01.0002	CSSu	1
				JBPX01.0002	CSSuTCot	2
				JBPX01.0002	SSuGen	4
	B (unrelated)	16	0-84	JBPX01.0002	AAuCxFox	1
	Extra not on tree	103	N/A	JBPX01.0002	Cip(I)	1
				JBPX01.0002, JBPX01.0039	AAuCxFox	4
				JBPX01.0002, JBPX01.0039	S	2
				JBPX01.0039	A	1
				JBPX01.0039	CSuTCot	1
				JBPX01.0039	SSuGen	1
				JBPX01.0008	SSuTGen	3
				JBPX01.0039	T	1
				JBPX01.0039	NalCip(I)	1
1704MLTDK-1 Enteritidis	Before A	6		JEGX01.0004	None	0
Live Poultry	A	13	0-15	JEGX01.0004	None	0
	B	1	0-14	JEGX01.0021	None	0
	Between B&C	5		JEGX01.0021	None	0
	C	3	2-5	JEGX01.0005	None	0
	D	1	0-6	JEGX01.0005	None	0
	E	45		JEGX01.0005	A	2
				JEGX01.0005	ACSTAuAzm	1
				JEGX01.0005	ACip(I)	1
				JEGX01.0005	NalCip(I)	1
	After E	4		JEGX01.0005	AT	1
	Extra not on tree	26		JEGX01.0004	None	0
		25		JEGX01.0005	NalCip(I)	1
				JEGX01.0005	A	1
		2		JEGX01.0021	None	0
1704MLTDK-1 Hadar	A	32	0-19 (48-80 to B)	TDKX01.0049	AAuCxFox	1
Live Poultry				TDKX01.0049	SSuGen	3
				TDKX01.0049	SSuTGen	2
	B	3	0-4	TDKX01.0049	ST	3
	Extra not on tree	106	N/A	TDKX01.0049	AAuCxFox	1
				TDKX01.0049	CSSu	1
				TDKX01.0049	CSSuT	1
				TDKX01.0049	CSSuCot	2
				TDKX01.0049	CSSuCotNalCip(I)	1
				TDKX01.0049	S	1
				TDKX01.0049	SSuGen	2
				TDKX01.0049	SSuTGen	3
				TDKX01.0049	Su	1
				TDKX01.0049	SuCot	1
				TDKX01.0049	ST	1
				TDKX01.0049	T	1
1704MLTDK-1 I 4,[5],12:i	A	5	0-4 (0-49 to B)	JPXX01.1071	T	5
Live Poultry	B	3	0 (0-59 to C)	JPXX01.1071	None	0
	C	3	0-3 (0-59 to D)	JPXX01.1071	None	0
	D	3	0-10	JPXX01.1071	T	3
	Extra not on tree	9	N/A	JPXX01.1071	T	4
1704MLTDK-1 Indiana	A	4	0-4 (4-8 to clade B)	JFPX01.0049	SSuGen	1
Live Poultry	B	3	0-2	JFPX01.0049	None	0
	Extra not on tree	18	N/A	JFPX01.0049	None	0
1704MLTDK-1 Litchfield	No tree requested	17		JGXX01.0009	ACSSuT	2
Live Poultry				JGXX01.0009	ASTAuCxFox	1
				JGXX01.0009	CSSuTCot	2
				JGXX01.0009	ST	4
				JGXX01.0009	SSuTGen	1
				JGXX01.0009	T	1

Continued next page

APPENDIX 1. RESISTANCE PROFILES FOR OUTBREAKS OF ZOONOTIC SALMONELLOSIS BY WHOLE GENOME SEQUENCING TREE CLADE DESCRIPTIONS, 2015-2018 (CONT.)

Outbreak	WGS Tree Clade	Human Isolates	Distance within Clade	PFGE Pattern	Resistance Pattern	Isolates with AMR (#)
1704MLTDK-1 Mbandaka Live Poultry	A	26	0-6 (54-88 to B)	TDRX01.0011	A	1
				TDRX01.0011	AAuCxFox	1
				TDRX01.0011	SSuGen	1
	B (unrelated)	4	0-4	TDRX01.0067	None	0
	Extra not on tree	40	N/A	TDRX01.0011	AAuCxFox	2
				TDRX01.0067	S	1
				TDRX01.0067	SSuGen	1
				TDRX01.0067	SSuTGen	1
1704MLTDK-1 Typhimurium Live Poultry	A	30	0-5	JPXX01.0033	AAuCxFox	1
				JPXX01.0033	SSuTGen	1
				JPXX01.0033	SSuGen	4
	A	4		JPXX01.5209	ASuCot	4
	Between A&B	4		JPXX01.0033	None	0
	B (unrelated)	7	0-2	JPXX01.0351	None	0
	Extra not on tree	98		JPXX01.0033	ACSSu	1
				JPXX01.1289	ACSSuTCotNalCip(I)GenAzm	1
				JPXX01.5209	ASuCot	1
				JPXX01.0033	AAuCxFox	4
				JPXX01.0033	CS	1
				JPXX01.0033	CSSu	1
				JPXX01.0033	SSuGen	8
				JPXX01.0033	SSuTGen	5
				JPXX01.0033	SSuTCot	1
				JPXX01.0033	T	1
					Total Resistant Isolates	142
					Resistant isolates on WGS tree	65
					Extra isolates not on tree	77
1701MLJRF-1 Turtles	A	15	1-14	JRFX01.0018	AAuCxFox	1
	Outlier	2		JRFX01.0018	None	0
	Extra not on tree	5	N/A	JRFX01.0018	AAuCxFox	1
1706MLJRF-1 Turtles	A	35	0-18	JRFX01.0018	None	0
	Extra not on tree	12	N/A	JRFX01.0018	NalCip(I)	1
1707 MLJKX-1 Snakes	A	2	2	JKXX01.0861	NalCip	1
				JKXX01.0861	NalCip(I)	1
	B	3	23-24	JKXX01.0861	NalCip(I)	1
				JKXX01.0861	CNalCip	1
				JKXX01.0861	CTNalCipAzm	1
	Extra not on tree	6	N/A	TDRX01.0005	None	0
					Total Resistant Isolates	8
					Resistant isolates on WGS tree	6
					Extra isolates not on tree	2
1608WIJF6-1 Dairy Calves	A	11	0-21 (15-39 to next)	JF6X01.0523,JFX01.0646	ACSSuTAuCxFoxCip(I)	3
				JF6X01.0523	ACSSuTAuCxFoxCip(I)Kan	5
				JF6X01.0523	ASSuTAuCxFoxCotNalCip	2
				JF6X01.0523	ASSuTAuCxFoxCip(I)KanFos	1
	B	4	2-13 (15-20 to next)	JF6X01.0590	ACSSuTAuCxFoxCip(I)	1
				JF6X01.0590	ACSSuTAuCxFoxCotNalCip	1
				JF6X01.0590	ACSSuTAuCxFoxCip(I)Kan	2
	Outlier	N/A		JF6X01.0824	N/A	N/A
	Extra not on tree	33	N/A	JF6X01.0523	ACSSuTAuCxFoxCip(I)	1
				JF6X01.0523, 0590, 0805, 0811	ACSSuTAuCxFoxCip(I)Kan	23
				JF6X01.0805	ACSSuTAuCxFoxCip(I)KanFos	1
				JF6X01.0523	ACSSuTAuCxFoxCotNalCip	2
				JF6X01.0646	ASSuTAuCxFoxCip	1
				JF6X01.0523, JF6X01.0811	ASSuTAuCxFoxCotCip	2
				JF6X01.0523	ASSuTAuCxFoxCotNalCip	1
				JF6X01.0523	CSSuTCip(I)Kan	1
				JF6X01.0824	None	0
					Total Resistant Isolates	47
					Resistant isolates on WGS tree	15
					Extra isolates not on tree	32
1801COJEG-1 Guinea Pigs	A	7	0-18	JEGX01.0021	None	0
	B	2	1	JEGX01.0021	SSuCot	1
	Extra not on tree	1	N/A	JEGX01.0021	None	0
					Total Resistant Isolates	1
					Resistant isolates on WGS tree	1
					Extra isolates not on tree	0

REFERENCES

Aguilar, F. (1967). Scanning the business environment. New York (N.Y.): Macmillan.

American Veterinary Medical Association [AVMA]. (2018). Antimicrobial stewardship definition and core principles. Retrieved from https://www.avma.org/KB/Policies/Pages/Antimicrobial-Stewardship-Definition-and-Core-Principles.aspx

Basler, C., Nguyen, T., Anderson, T. C., Hancock, T., & Behravesh, C. (2016). Outbreaks of Human *Salmonella* Infections Associated with Live Poultry, United States, 1990–2014. *Emerging Infectious Diseases*, 22(10), 1705-1711.

Brown, A. C., Grass, J. E., Richardson, L. C., Nisler, A. L., Bicknese, A. S., & Gould, L. H. (2016). Antimicrobial resistance in Salmonella that caused foodborne disease outbreaks: United States, 2003-2012. *Epidemiology and infection*, *145*(4), 766-774.

Carlton, E., Holsinger, J., Riddell, M., & Bush, H. (2015). Full-Range Public Health Leadership, Part 1: Quantitative Analysis. *Frontiers In Public Health*, *3*. doi: 10.3389/fpubh.2015.00073.

Centers for Disease Control and Prevention [CDC]. (n.d.). *Don't play chicken with your health*. Retrieved from https://www.cdc.gov/healthypets/resources/15_261877-b_dont_play_chicken_with_your_health_508compliant.pdf

CDC. (n.d.). Outbreak Infographic. Retrieved from https://www.cdc.gov/foodsafety/outbreaks/pdfs/outbreak-infographic.pdf

CDC. (n.d.). *Salmonellosis* | *Summary* | *NNDSS*. Retrieved from https://wwwn.cdc.gov/nndss/conditions/salmonellosis/

CDC. (n.d.). Salmonellosis | 2017 Case Definition. Retrieved from https://wwwn.cdc.gov/nndss/conditions/salmonellosis/case-definition/2017/

CDC. (1999). Ten great public health achievements—United States, 1900–1999. *MMWR Morbidity And Mortality Weekly Report*, 48, 241–243.

CDC. (2013). Antibiotic Resistant Threats in the United States, 2013. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC.

CDC. (2014). Core Elements of Hospital Antibiotic Stewardship Programs. Atlanta, GA: US Department of Health and Human Services, CDC. Retrieved from https://www.cdc.gov/antibiotic-use/healthcare/pdfs/core-elements.pdf

CDC. (2015). The Core Elements of Antibiotic Stewardship for Nursing Homes. Atlanta, GA: US Department of Health and Human Services, CDC. Retrieved from http://www.cdc.gov/longtermcare/index.html

CDC. (2015). *Timeline for Reporting Cases of Salmonella Infection | Salmonella | CDC*. Retrieved from https://www.cdc.gov/salmonella/reporting-timeline.html

CDC. (2016). *About FoodNet | FoodNet | CDC*. Retrieved from https://www.cdc.gov/foodnet/about.html

CDC. (2016). *Frequently Asked Questions | About | PulseNet | CDC*. Retrieved from https://www.cdc.gov/pulsenet/about/faq.html#future

CDC. (2016). *Outbreak Detection | PulseNet | CDC*. Retrieved from https://www.cdc.gov/pulsenet/outbreak-detection/index.html

CDC. (2017). Implementation of Antibiotic Stewardship Core Elements at Small and Critical Access Hospitals. Retrieved from https://www.cdc.gov/antibiotic-use/healthcare/pdfs/core-elements-small-critical.pdf

CDC. (2017). *Surveillance Case Definition | Salmonella*. Retrieved from https://wwwn.cdc.gov/nndss/conditions/salmonellosis/case-definition/2017/

CDC. (2018). *About NORS | National Outbreak Reporting System (NORS) | CDC*. (2018). Retrieved from https://www.cdc.gov/nors/about.html

CDC. (2018). *Enteric Disease Laboratory Branch Isolate Submission Table*. Retrieved from https://www.cdc.gov/ncezid/dfwed/pdfs/edlb-isolate-submission-table-508c.pdf

CDC. (2018). *Enteric Zoonoses Activity | ORPB | DFWED | NCEZID | CDC*. Retrieved from https://www.cdc.gov/ncezid/dfwed/orpb/eza.html

CDC. (2018). *Information for Healthcare Professionals | Salmonella | CDC*. Retrieved from https://www.cdc.gov/salmonella/general/technical.html

CDC. (2018). *Multistate Outbreaks of Salmonella Infections Linked to Contact with Live Poultry in Backyard Flocks, 2018 (Final Update)*. Retrieved from https://www.cdc.gov/salmonella/backyard-flocks-06-18/index.html

CDC. (2018). *Multistate Outbreak of Salmonella Agbeni Infections Linked to Pet Turtles, 2017 (Final Update)*. Retrieved from https://www.cdc.gov/salmonella/agbeni-08-17/index.html

CDC. (2018). *National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) | NARMS | CDC*. Retrieved from https://www.cdc.gov/narms/index.html

CDC. (2018). *National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Surveillance Report for 2015 (Final Report)*. Retrieved from https://www.cdc.gov/narms/reports/annual-human-isolates-report-2015.html

CDC. (2018). *Questions and Answers | Salmonella | CDC*. (2016). Retrieved from https://www.cdc.gov/salmonella/general/index.html

CDC. (2018). *Salmonella infection | Healthy Pets, Healthy People*. Retrieved from https://www.cdc.gov/healthypets/diseases/salmonella.html

CDC. (2018). *Salmonella | Surveillance*. Retrieved from https://www.cdc.gov/salmonella/reportspubs/surveillance.html

CDC. (2018). *US Outbreaks of Zoonotic Diseases Spread between Animals & People | Healthy Pets, Healthy People | CDC*. Retrieved from https://www.cdc.gov/healthypets/outbreaks.html

CDC. (2018). *What Exactly is Antibiotic Resistance?* Retrieved from https://www.cdc.gov/drugresistance/about.html

Community Tool Box. (2017). *Chapter 7. Encouraging Involvement in Community Work | Section 8. Identifying and Analyzing Stakeholders and Their Interests*. Retrieved from http://ctb.ku.edu/en/table-of-contents/participation/encouraging-involvement/identify-stakeholders/main

Day, M., Shickle, D., Smith, K., Zakariasen, K., Moskol, J., & Oliver, T. (2014). Training public health superheroes: five talents for public health leadership. *Journal Of Public Health*, 36(4), 552-561.

DeSalvo, K., O'Carroll, P., Koo, D., Auerbach, J., and Monroe J. (2016) "Public Health 3.0: Time for an Upgrade", *American Journal of Public Health*, 106(4), 621-622.

Food and Drug Administration. (2018). *Supporting Antimicrobial Stewardship In Veterinary Settings*. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/UCM620420.pdf.

International Association of Public Participation [IAP2]. (2014). IAP2's Public Participation Spectrum. Retrieved from https://cdn.ymaws.com/www.iap2.org/resource/resmgr/foundations_course/IAP2_P2_Spectrum_FINAL.pdf.

Karp, B., Tate, H., Plumblee, J., Dessai, U., Whichard, J., & Thacker, E. et al. (2017). National Antimicrobial Resistance Monitoring System: Two Decades of Advancing Public Health Through Integrated Surveillance of Antimicrobial Resistance. *Foodborne Pathogens And Disease*, *14*(10), 545-557.

Kouzes, J., & Posner, B. (2012). *The Leadership Challenge* (5th ed.). San Francisco: John Wiley & Sons, Incorporated.

McDermott, P., Tyson, G., Kabera, C., Chen, Y., Li, C., & Folster, J. et al. (2016). Whole-Genome Sequencing for Detecting Antimicrobial Resistance in

Nontyphoidal Salmonella. *Antimicrobial Agents And Chemotherapy*, *60*(9), 5515-5520. doi: 10.1128/aac.01030-16

Montgomery, M., Robertson, S., Koski, L., Salehi, E., Stevenson, L., & Silver, R. et al. (2018). Multidrug-Resistant Campylobacter jejuni Outbreak Linked to Puppy Exposure — United States, 2016–2018. *MMWR. Morbidity And Mortality Weekly Report*, *67*(37), 1032-1035.

RESOLVE. (2014). The High Achieving Governmental Health Department in 2020 as the Community Chief Health Strategist. Washington, DC: RESOLVE. Retrieved from http://www.resolv.org/site-healthleadershipforum/files/2014/05/The-High-Achieving-Governmental-Health-Department-as-the-Chief-Health-Strategist-by-2020-Final1.pdf

Robertson, S., Burakoff, A., Stevenson, L., Tompkins, B., Patel, K., & Tolar, B. et al. (2018). Notes from the Field: Recurrence of a Multistate Outbreak of Salmonella Enteritidis Infections Linked to Contact with Guinea Pigs — Eight States, 2015–2017. *MMWR. Morbidity And Mortality Weekly Report*, *67*(42), 1195-1196.

Sanchez, G., Fleming-Dutra, K., Roberts, R., & Hicks, L. (2016). Core Elements of Outpatient Antibiotic Stewardship. *MMWR. Recommendations And Reports*, *65*(6), 1-12. doi: 10.15585/mmwr.rr6506a1

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., et al. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging infectious diseases*, *17*(1), 7-15.

Shane, A., Mody, R., Crump, J., Tarr, P., Steiner, T., & Kotloff, K. et al. (2017). 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. *Clinical Infectious Diseases*, *65*(12), e45-e80.

Turtles intrastate and interstate requirements, 21 CFR 1240.62 (2018).

World Health Organization. Ad-hoc Interagency Coordination Group. Retrieved from: www.who.int/antimicrobial-resistance/interagency-coordination-group/en/.

World Health Organization. (2018). *Antibiotic resistance*. Retrieved from http://www.who.int/en/news-room/fact-sheets/detail/antibiotic-resistance.

World Health Organization. (2018). *Antimicrobial resistance*. Retrieved from http://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance.

Yukl, G. & Uppal, N. (2012). *Leadership in Organizations.* London: Pearson.