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Antimicrobial Resistance and Epidemiology of Salmonella serotype Kentucky infections—United States, 2009–2022

Caroline Snyder

Antimicrobial Resistance and Epidemiology of *Salmonella* serotype Kentucky infections—United States, 2009–2022

by

Caroline Snyder

Under the Direction of Kevin M. Maloney, PhD, MPH

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ABSTRACT

Salmonella enterica serotype Kentucky (*Salmonella* Kentucky) is the predominant serotype found in retail chicken products (U.S. Food and Drug Administration, 2022). Despite its high likelihood to be found in chicken, *Salmonella* Kentucky has caused few human illnesses relative to other *Salmonella* serotypes. Since the 1980s, a multidrug-resistant (MDR) *Salmonella* Kentucky strain has been disseminating from Egypt to other countries. This MDR strain is characterized by resistance determinants including a mutation that confers resistance to fluoroquinolones like ciprofloxacin; ciprofloxacin-resistant *Salmonella* are classified as high priority pathogens on the World Health Organization's (WHO) list of priority resistant bacteria. CDC monitors U.S. cases of MDR *Salmonella* Kentucky.

This study aims to characterize *Salmonella* Kentucky isolates from U.S. surveillance systems to describe antimicrobial resistance determinants found in *Salmonella* Kentucky between 2009–2022. We also analyze relevant epidemiological data associated with MDR *Salmonella* Kentucky cases. There were 7,329 isolates including of which 5,209 were chicken, 476 were other food, and 311 were human. Significant findings include that 66% of human isolates were ciprofloxacin resistant. This MDR *Salmonella* Kentucky strain is a growing public health concern in the United States.

INDEX WORDS: *Salmonella, Salmonella* serotype Kentucky, Antimicrobial Resistance, Ciprofloxacin Resistance, Multidrug Resistance

Antimicrobial Resistance and Epidemiology of *Salmonella* serotype Kentucky infections—United States, 2009–2011

by

CAROLINE SNYDER

Committee Chair: Kevin M. Maloney, PhD, MPH

Committee: Louise Francois Watkins, MD, MPH

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Office of Graduate Services

School of Public Health

Georgia State University

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DEDICATION

I dedicate this thesis to the most important people in my life, who have been my constant support and motivation throughout my academic journey. To my mom and dad, thank you for your unconditional love and unwavering belief in my abilities. You have always encouraged me to pursue my dreams and have provided me with the tools and resources to achieve them. To my sisters, thank you for being my best friends and for always cheering me on. Your support has been invaluable to me. To my grandparents, thank you for your unwavering love and wisdom. Your stories and life experiences, and constant motivation have inspired me to pursue my dreams. To my teachers, thank you for your guidance, wisdom, and patience. You have not only imparted knowledge but have also instilled in me a love for learning. This thesis is a testament to the love, guidance, and support of all these amazing people in my life.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
CDC	Centers for Disease Control and Prevention
cgMLST	Core Genome Multi-Locus Sequence Typing
NARMS	National Antimicrobial Resistance Monitoring System
NCBI	National Center for Biotechnology Information
NORS	National Outbreak Reporting System
LEDS	Laboratory-Based Enteric Disease System
MDR	Multidrug Resistant
QRDR	Quinolone Resistance Determining Region
REP Strain	Reoccurring Emerging and Persisting Strain
Salmonella Kentucky	*Salmonella* serotype Kentucky
ST	Sequence Type
WGS	Whole Genome Sequencing
WHO	World Health Organization
U.S.	United States

CHAPTER 1: INTRODUCTION

1.1 Salmonella Infection

Salmonellosis, caused by infection with *Salmonella* bacteria, is attributable to 1.35 million illnesses and 400 deaths each year in the United States (*Salmonella* Annual Report, 2016, Collier et al., 2021). It is estimated that illness due to foodborne pathogens costs the U.S. public $15.5 billion in economic burden each year (Batz et al., 2021). The Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes more foodborne illnesses than any other bacteria (CDC "Food Safety", 2022). Symptoms of *Salmonella* infection include diarrhea, bloody diarrhea, fever, stomach cramps, nausea, vomiting, and headaches (CDC *Salmonella* Symptoms, 2019). The incubation period can vary from six hours to six days after infection and the duration of illness typically lasts four to seven days (CDC *Salmonella* Symptoms, 2019). While not all *Salmonella* infections require treatment, it is recommended that persons experiencing severe salmonellosis be treated with antimicrobials (Shane et al., 2017). *Salmonella* can acquire resistance to antimicrobials through the acquisition of resistant genes or plasmids. Multidrug-resistant (MDR) *Salmonella,* defined as *Salmonella* resistant to one or more antimicrobial agents in three or more antimicrobial classes, is a public health concern because it may cause more severe health outcomes (Parisi et al., 2018). The use of antimicrobials in treatment for patients or agricultural practices creates a selective pressure for *Salmonella* bacteria towards acquiring antimicrobial resistance factors (Parisi et al., 2018).

1.2 Salmonella Kentucky

Ten percent of foodborne *Salmonella* illnesses in the United States can be attributed to chicken (Batz et al., 2021). *Salmonella* serotype Kentucky (*Salmonella* Kentucky) is the predominant serotype found in retail chicken products (U.S. Food and Drug Administration,

2022). Despite its' high likelihood to be found in chicken, *Salmonella* Kentucky has caused few human illnesses relative to other *Salmonella* serotypes; of the 477,861 *Salmonella* culture-confirmed cases submitted to the Laboratory Based Enteric Disease System, only 0.2% (1,026) were *Salmonella* Kentucky (*Salmonella* Annual Report, 2016). Only five *Salmonella* Kentucky outbreaks have been reported to the National Outbreak Reporting System since 1998 (Tate et al., 2022). Studies have found that *Salmonella* Kentucky is less harmful to humans because it lacks virulent genes associated with more severe human illness, which explains why it is often found in chicken but not in human incidence reports (Tasmin et al., 2017). However, a global strain of multidrug-resistant (MDR) *Salmonella* Kentucky has emerged in recent years and created a pathway for *Salmonella* Kentucky to cause more human illnesses through the acquisition of more virulent genes (Le Hello et al., 2013).

A 1989 report from Egypt described a strain of *Salmonella* Kentucky that acquired a variant of *Salmonella* genomic island SG1-k which conferred resistance to multiple antibiotics including ampicillin, streptomycin, gentamycin, sulfonamides, and tetracycline (Vasquez et al., 2021). As it spread, the strain also acquired resistance to ciprofloxacin through a triple mutation in the quinolone-resistance-determining region (QRDR) (Wang et al., 2021). Ciprofloxacin resistant *Salmonella* are classified as high priority pathogens on the World Health Organization's (WHO) list of priority resistant bacteria (Biggel et al., 2022). This strain disseminated into Northern, Southern, and Western Africa and spread to Asia and Europe (Vasquez et al., 2021). The strain has been found in chicken flocks of many countries including France, England, China, multiple African countries, and most recently Canada (Tasmin et al., 2017, Haley et al., 2019, Sousa Sariva et al., 2022, Copian et al., 2020). Furthermore, it is hypothesized that this strain can be found in high levels among countries where there is uncontrolled administration of

fluoroquinolones in the poultry industry (Tate et al., 2022). While the global MDR strain has not yet been found in chicken from the U.S., it has been documented in human cases (Tate et al., 2022).

Salmonella Kentucky is a polyphyletic serotype, indicating that it consists of multiple sequence types (ST) that do not share a common ancestor (Tate et al., 2022). This can result in highly divergent clades of *Salmonella* Kentucky (Tate et al., 2022). Sequence type (ST) is a genomic characterization that describes the seven house-keeping genes that a bacterium has; it is typically denoted as ST###. The globally distributed strain described above has been designated as ST198. Previous work by Tate et al. (2022) found that the majority of chicken isolates in the United States collected through the National Antimicrobial Resistant Monitoring System (NARMS) are ST152. Tate et al. (2022) also describes that ST198 *Salmonella* Kentucky isolates collected from humans can be divided into two phylogenetic clades: a fluoroquinolone resistant clade and a fluoroquinolone susceptible clade. Since 2020, CDC has monitored U.S. cases of the fluoroquinolone resistant clade of ST198, hereafter Strain A, highlighted in Tate et al. (2022) which has similar resistant markers to that of the global MDR strain. CDC has monitored this clade to assess the prevalence of Strain A within the United States

We analyze *Salmonella* Kentucky isolates from U.S. surveillance systems to describe antimicrobial resistance of *Salmonella* Kentucky isolates in the United States reported between 2009 and 2022. Furthermore, we characterize the available epidemiology of cases of Strain A in the United States.

CHAPTER 2: METHODS

2.1 Data Sources

Data sources included PulseNet, CDC's National Antimicrobial Resistance Monitoring System (NARMS), and the National Center for Biotechnology Information (NCBI) Pathogen Detection Pipeline. Characteristics assessed in this analysis include the isolate's sequence type, antimicrobial resistance determinants, demographic characteristics of human cases, patient travel history collected from human cases, and food history collected from human cases.

2.1.1 PulseNet

PulseNet is the national laboratory subtyping network for foodborne outbreaks (Tolar et al., 2019). PulseNet is an isolate-based, passive surveillance system that consists of 82 state and local public health laboratories and spans the U.S. (Tolar et al., 2019). PulseNet was established in 1996 and used pulse-field gel electrophoresis until transferring to whole genome sequencing (WGS) in 2019 as the primary subtyping laboratory method (Tolar et al., 2019); before 2019, WGS was performed on some *Salmonella* isolates at the discretion of individual public health laboratories. PulseNet participating laboratories submitted WGS results and limited epidemiological information for clinical isolates from multiple organisms including *Salmonella* Kentucky (Tolar et al., 2019). Non-human isolates could also be shared to PulseNet and compared with human isolates by molecular subtyping techniques, such as core-genome multi-loci sequence typing (cgMLST). Even though WGS simplified and enhanced sequencing efficiency for PulseNet participants, many participating laboratories lacked the resources to sequence 100% of the *Salmonella* isolates they received. PulseNet uses a customized data analysis and management software named BioNumerics, developed by Applied Maths, to house isolate subtyping information (Tolar et al., 2019).

2.1.2 National Antimicrobial Resistance Monitoring System (NARMS)

NARMS was established in 1996 as a collaboration among CDC, U.S. Food and Drug Administration (FDA), United States Department of Agriculture (USDA) and state and local public health departments. NARMS at CDC is an isolate-based passive surveillance system that determines antimicrobial resistance for *Campylobacter, Shigella, E. coli* O157, *Salmonella,* and *Vibrio* from two different methods. NARMS scientists analyzed WGS results from isolates uploaded to PulseNet to determine a *Salmonella* isolate's predictive resistance. In addition, NARMS scientists performed antimicrobial susceptibility testing (AST) on *Salmonella* isolates received from public health partners.

2.1.3 National Center for Biotechnology Information (NCBI) Pathogen Detection Pipeline

The National Center for Biotechnology Information (NCBI)'s pathogen detection pipeline is a public platform that integrates bacterial and fungal pathogen genomic sequences, including *Salmonella,* from ongoing surveillance and research efforts. Raw sequence data of good quality sequences uploaded to PulseNet were also imported to NCBI and linked back to the PulseNet sequences using an isolate identifier (Tolar et al., 2019). Unlike PulseNet, NCBI's platform uses single-nucleotide polymorphisms (SNPs) as the primary genomic subtyping characterization method. NCBI uses AMR FinderPlus to determine an isolate's genotypic resistance genes (Bortolaia et al., 2020).

2.1.4 Epidemiological Data

When isolates were identified as belonging to Strain A by PulseNet, CDC requested and collected epidemiological information. For cases detected within 60 days, CDC provided a dedicated interview form. For older cases, CDC requested any available information, typically from the data collection tool specific to that public health jurisdiction. These two methods are not

standardized, but for the purposes of this study, information from both tools was extracted and harmonized. We supplemented missing demographic information with data from PulseNet.

2.2 Strain A Characterization

PulseNet identified isolates for Strain A if the isolate had a serotype of "Kentucky," ST of ST198, and if it belonged within the clade identified as 198.2 using cgMLST, which characterizes isolates by their core-genome content. Tate et al. (2022) described two clades of ST198 *Salmonella* Kentucky isolates in the United States: a fluoroquinolone-susceptible clade (198.1) of agricultural sources, and a fluoroquinolone-resistant clade (198.2) of mostly human isolates corresponding to the global MDR *Salmonella* Kentucky strain (including the QRDR mutation). CDC followed the fluoroquinolone-resistant clade to monitor for increased prevalence and actionable public health steps.

2.3 Antimicrobial Resistance

NARMS received every 20th *Salmonella* isolate from 54 state and local public health laboratories and additional isolates submitted as part of outbreaks and special studies for AST by broth microdilution. AST is a laboratory procedure performed on *Salmonella* isolates to determine resistance. AST determines the lowest concentration of an antibiotic that will inhibit growth of *Salmonella* in culture (the minimum inhibitory concentration [MIC]); the MIC-value is used to label the bacteria as susceptible, intermediate, or resistant. AST using broth microdilution is considered the gold standard for antimicrobial resistance prediction for laboratories performing AST (McDermott et al., 2016). WGS results from PulseNet are also received by NARMS and used to predict a *Salmonella* isolate's phenotypic resistance. Studies have shown that WGS can routinely be used as the primary subtyping method and can accurately predict resistance based on the presence or absence of resistance determinants; one study found that in

640 *Salmonella* isolates from 43 different serotypes, resistance genotypes and phenotypes correlated in 99.0% of cases (McDermott et al., 2016).

For isolates that were analyzed by NARMS, we used AST results when available but isolates without AST results were supplemented with the isolate's predictive resistance based on WGS. For isolates that were not analyzed by NARMS, we supplemented resistance information with resistant determinants found on NCBI's pathogen detection pipeline. Resistance genes predicted by NCBI's AMRFinderPlus were evaluated to predict the isolate's resistance phenotype.

2.4 Definitions

Strain A. We defined Strain A as isolates that were identified within the ST198.2 clade within PulseNet based on cgMLST.

Travel-associated. We considered cases to be travel-associated if cases reported travelling out of the country in the 6 months prior to illness onset. This data was collected from case report forms and epidemiological case information sent to NARMS by public health partners. When information was not available, the case was not counted as having travel exposures. Only cases that reported specifically that they did or did not travel were included in the analysis.

Food-associated exposure. We considered cases to have food-exposure if cases reported eating suspected food 7 days prior to illness onset. This data was collected from case report forms and epidemiological case information sent to NARMS by public health partners.

Multidrug resistance. We defined multidrug resistance as non-susceptible or resistant to 1 or more agents in 3 or more antimicrobial resistance classes as defined by the Clinical & Laboratory Standards Institute (Clinical Laboratory Standards Institute, 2023).

Ciprofloxacin resistance and non-susceptibility. We defined an isolate with AST results as ciprofloxacin non-susceptible if the isolate's results were R (resistant) or I (intermediate). We defined an isolate with WGS predictive resistance results as ciprofloxacin non-susceptible if it had a predictive resistance of decreased susceptible (DS), or R (resistant). Within the NARMS database, an isolate is determined as having decreased susceptibility to ciprofloxacin for WGS predictive resistance when it has at least one quinolone resistant mechanism. We defined an isolate with NCBI resistance results as ciprofloxacin non-susceptible if it had one or more resistance determinants that confers resistance for ciprofloxacin. A single resistance determinant, or presence of single ciprofloxacin resistant gene or mutation, typically correlates with a ciprofloxacin MIC in the intermediate range of 0.12 to 0.5 ug/ml, while two or more resistance determinants correlate with an MIC of ≥1 ug/ml in the resistance range (NARMS Now: Human Data, 2023). Unlike other antibiotics, ciprofloxacin has a true intermediate MIC range. Clinical outcome data suggests intermediate resistance to ciprofloxacin has been associated with treatment failures. CDC recommends to only use ciprofloxacin if the *Salmonella* strain is fully susceptible. For the purposes of analysis, we considered ciprofloxacin non-susceptible isolates to be resistant.

Resistant. For antimicrobials other than ciprofloxacin, we defined an as isolate as resistant if an isolate's AST results were "R" (resistant), if an isolate's predictive WGS results were determined as "R" (resistant), or if an isolate's NCBI results included one or more resistance determinants that confers resistance to that antimicrobial. For isolates that had supplemental resistance information from NCBI, streptomycin resistant strains were defined as "R" (resistant) if they had both the *aph(3'')-Ib* and *aph(6)-Id* genes present or another single streptomycin resistant gene.

2.5 Data Analysis

We requested records for all *Salmonella* Kentucky isolates with WGS results that were uploaded to PulseNet and that had isolation dates between the years of 2009–2022. We limited our analysis to sequenced *Salmonella* Kentucky isolates with records in both NARMS and PulseNet surveillance systems and excluded records that did not have matching serotype and isolate identifying information between systems. We matched *Salmonella* Kentucky isolates from NARMS and PulseNet surveillance systems using WGS IDs, an isolate identifier that is systematically assigned to isolates once they are sequenced and uploaded to PulseNet. Serotype, sequence type (ST), and antigen formula are determined by BioNumerics' calculation engine within PulseNet (Tolar et al., 2019). We also excluded isolates that were known duplicates to other isolates.

We performed a descriptive epidemiological study. We compared proportions by isolate source type and ST and assessed antimicrobial resistance patterns for all *Salmonella* Kentucky isolates. We compared Strain A human isolates to other human strains by analyzing patient age, patient sex, and distribution of patient in the United States through a chi-square analysis and p-value (values of <0.05 were considered statistically significant). We assessed travel history, common food exposures, and other epidemiological information of Strain A cases. All statistical analyses and data merging were performed in SAS Studio, a cloud-based online version of SAS.

CHAPTER 3: RESULTS

3.1 Salmonella Kentucky

There were 7,753 *Salmonella* Kentucky isolates with records in the PulseNet and NARMS surveillance systems between the years of 2009–2022. Of these, 3,378 (44%) isolates were analyzed by NARMS and 4,377 (56%) were not analyzed by NARMS. Of the 4,377 records without resistance results analyzed in NARMS, 3,951 (90%) had matching serotypes and isolate identifiers between PulseNet and NARMS and were supplemented with NCBI resistance data. In total, there were 7,329 isolates included in the analysis: 6,167 (84%) food-chicken isolates, 476 (7%) animal isolates, 358 (5%) food other isolates, 311 (4%) human isolates, and 16 (<1%) environmental sample isolates (Table 1).

Table 1. Summary of Sequence Type and Source Type

	Sequence Types					
	ST152	ST198	ST314	ST Other	Strain A	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Source type						
Human	59 (1%)	218 (65%)	25 (50%)	4 (2%)	195 (95%)	311 (4%)
Animal	285(5%)	32 (10%)	8 (16%)	4 (2%)	0 (0%)	476 (7%)
Environmental	11 (<1%)	5 (2%)	0 (0%)	0 (0%)	0 (0%)	16 (0.2%)
Food (Chicken)	4,634 (89%)	6 (2%)	14 (29 %)	183 (91%)	0 (0%)	6,167 (84%)
Food (Other)	220 (4%)	71 (21%)	71 (%)	10 (5%)	10(5%)	358 (5%)
Total	5,209 (100%)	333 (100%)	348 (100%)	201 (100%)	205 (100%)	7,329 (100%)

Table 1. Percent indicates column percent. Source type and sequence type were taken from PulseNet. Not all isolates had sequence type information. Total column indicates that total number of isolates used in study even if ST information is missing. All Strain A isolates are ST198.

3.2 Source Type Human Isolates

Of 311 human isolates, 218 (71%) were ST198, 59 (19%) were ST152, 25(8%) were ST314, 3 (2%) were ST696, and 1 (<1%) was ST118. Of the isolates that were ST198, 195 (64%) were classified as Strain A. The median age of all human patients was 48 years, with a mean of 44 and standard deviation of 24.58. Ages varied from 3 months to 89 years of age. For patient sex, 168 (55%) were female, 121 (39%) were male, and 22% (7%) had no patient sex data (Table 2). Most human isolates were reported from NY (39, 12%), CA (38, 12%) and TX (25, 8%). Figure 1 shows the distribution of human *Salmonella* Kentucky isolates in the United States.

Table 2. Patient Age, Patient Sex, and Reporting United States Region for Human Isolates, Strain A vs. Other *Salmonella* Kentucky Human Strains (n=311)

	Strain A (n=195) N (%)[1]	Other Human Strains (n=116) N (%)[1]	Chi Square P-Value[2]
Patient Age			<0.01
0– 4	13 (7%)	19 (16%)	
5–9	2 (1%)	0 (0%)	
10–19	8 (4%)	11 (10%)	
20–29	31 (17%)	5 (4%)	
30–59	76 (41%)	35 (30%)	
60+	53 (28%)	44 (38%)	
Unknown	12 (6%)	2 (2%)	
Patient Sex			0.28
Male	80 (41%)	41 (35%)	
Female	104 (54%)	64 (56%)	
Unknown	11 (9%)	9 (5%)	
Reporting United States Region			<0.01
Northeastern	73 (37%)	33 (28%)	
Southeastern	23 (12%)	9 (8%)	
North Central	37 (19%)	15 (13%)	
Central	26 (13%)	35 (30%)	
Western	36 (18%)	24 (21%)	

1. Percent indicates the percentage of total column.
2. Statistical significance is defined as p<0.05.

Figure 1. Distribution of reported *Salmonella* Kentucky in the United States 2009–2022, n=311

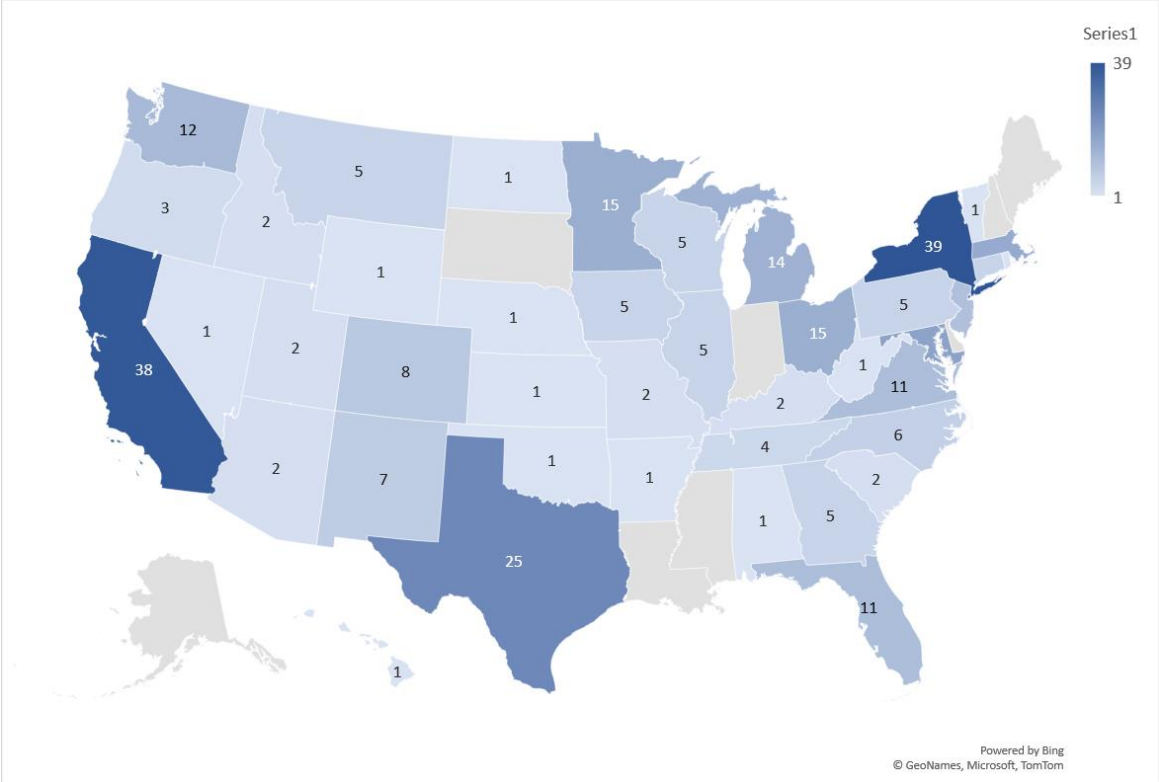

Figure 1. Distribution of reported *Salmonella* Kentucky human cases in the United States. State count indicates the reporting state laboratory.

In total, 57 human isolates had no resistance determinants and were pan-susceptible, all were of other human *Salmonella* Kentucky strains. Of all human isolates, 206 (66%) were resistant to ciprofloxacin; 195 of 206 (95%) were Strain A. For other human isolates not classified as Strain A, only 9 (7%) isolates were defined as MDR, while 171 (87%) Strain A isolates were defined as MDR (Table 3).

Table 3. Antimicrobial Resistance of Human Isolates, Strain A Vs. Other Human Strains (n=311)

	Strain A (n=195, 63%)[1] N (%)[2]	Other Human Strains (n=116, 37%)[1] N (%)[2]
Amoxicillin-clavulanic acid	10 (5%)	4 (3%)
Ampicillin	169 (86%)	10 (9%)
Azithromycin	11 (5%)	1 (1%)
Cefoxitin	9 (4%)	4 (3%)
Ceftiofur	18 (9%)	4 (3%)
Ceftriaxone	18 (9%)	4 (3%)
Chloramphenicol	24 (12%)	4 (3%)
Ciprofloxacin[3]	195 (100%)	11 (5%)
Colistin	1 (1%)	0 (0%)
Gentamicin	126 (65%)	3 (2%)
Meropenem	0 (0%)	0 (0%)
Nalidixic acid	195 (100%)	5 (4%)
Streptomycin	141 (72%)	45 (38%)
Sulfisoxazole	148 (75%)	6 (5%)
Tetracycline	168 (86%)	39 (33%)
Trimethoprim-sulfamethoxazole	22 (11%)	3 (2%)
No predicted resistance	0 (0%)	57 (49%)
Resistance to ≥1 antimicrobial class[4]	195 (100%)	59 (51%)
Resistance to ≥3 antimicrobial classes[5]	171 (87%)	9 (7%)

1. Percent indicates total percent of all human isolates.
2. Percent indicates column percent.
3. Ciprofloxacin resistance is defined as having one or mechanisms for resistance, or as have decreased susceptibility or non-susceptibility to ciprofloxacin.
4. Defined as being resistant to 1 or more antimicrobial agents in 1 or more antimicrobial classes.
5. Defined as having resistance to 3 or more antimicrobial agents in 3 or more antimicrobial classes.

3.3 Strain A Isolates

Strain A consisted of 206 isolates, 10 taken from food, 195 taken from humans, and 1 with an unknown source. Strain A was first documented in CDC surveillance systems from a food isolate in 2009 and the first human isolate was recorded in 2011. Food source types included spice (n=5), snail (n=3), fish (1), and shellfish (1). The median age of humans within Strain A is 47 years, with a mean of 44 years and a standard deviation of 22.2 years. Compared with other human strains of *Salmonella* Kentucky, the differences between patient age were statistically significant (Table 2). There were 104 (53%) female patients, 80 (41%) male patients, and 11 (6%) patients with unknown sex (Table 2). The majority (n=73, 37%) of Strain A patients were reported from the North-Eastern region. Top reporting states for Strain A include CA (48, 25%), followed by NY (23, 12%), MD (14, 7%), and MA (13, 6%). When comparing the distribution of Strain A patients to other human strains patients in reporting states of the United States, there is a statistically significant difference among the two groups (p= 0.004) (Table 4). The majority (n=165, 85%) of isolates in Strain A were isolated after the year 2018 (Figure 2). For non-human strains, the majority (n=89, 77%) were also isolated after the year 2018. This finding is influenced by PulseNet converting to WGS in 2018 and only isolates with WGS results being included in the study.

Figure 2. Epidemiological Curve of Strain A Isolates by Source Type, 2009–2022 (n=205)

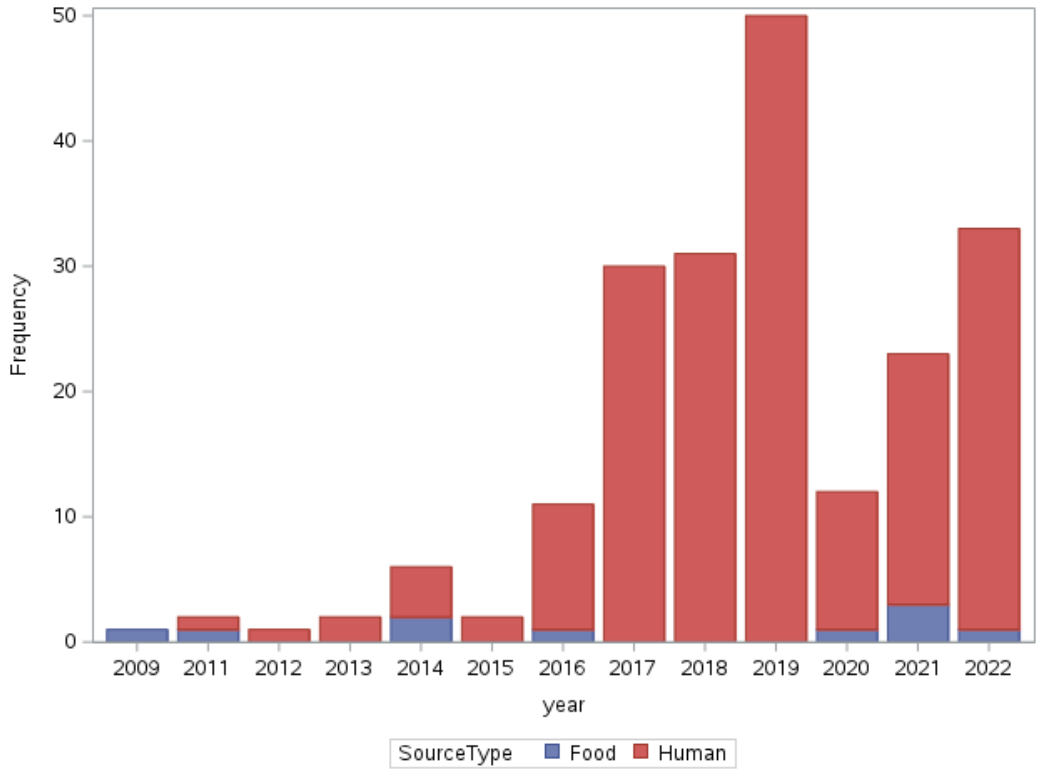

Figure 2. Epidemiological curve by year of all Strain A isolates. 195 human isolates and 10 food isolates are included.

3.3.1 Strain A Epidemiological Information

Of those with epidemiological information available, 24 of 25 (96%) patients reported having symptoms; nine of 25 (36%) patients were hospitalized. Of those who provided travel information, 24 of 36 (63%) reported yes to international travel prior to illness; 12 reported no travel. The most reported travel destinations included India (n=8), Egypt (n=3), and Pakistan (n=3). Two cases also reported having exposure on a cruise ship. For food exposures, 18 of 21 (85%) patients reported eating chicken, 12 of 21 (57%) reported eating beef, and 11 of 21 (52%) reported eating turkey. Ten of 20 (50%) patients reported animal contact. Seventeen cases reported having at least one underlying condition including renal disease, low blood sugar, type II diabetes, Crohn's disease, diffuse large B-cell lymphoma, and cancer treatment. There were

nine cases that reported occupation; of which, there was one correctional officer, one food handler, two health care providers, three retired persons, and one unemployed person (Table 4).

Table 4. Race, Travel History, and Occupation for Cases of Strain A

Race (D=24)[1]	
Asian (includes Pacific Islander or Native Hawaiian)	3
Black	2
American Indian	1
Multiracial	2
White Hispanic	1
White Non-Hispanic	15
Travel-Associated (D=36)[1]	
Yes	24
No	12
Destination Region	
Asia	14
Central America and the Caribbean	2
Europe	2
Middle East	3
North Africa	6
Sub-Saharan Africa	6
Occupation (D=9[1])	
Correctional Officer	1
Food-Handler	1
Health Care Provider	2
Retired	3
Unemployed	1

1. D represents the number of responses to question from epidemiological data received, not the total number of responses received.
***Some cases reported travelling to 1 or more countries during incubation period.**

3.4 Source Type Food-Chicken, Food-Other, Animal, and Environmental

3.4.1 *Source Type Food*

In total, there were 6,525 food isolates. There were 147 (2%) food isolates that did not indicate what type of food the isolate was sampled from. However, 6,167 (95%) were specified as chicken, 95 (1%) were from beef, and 35 (>1%) were from pork. There were 1,359 (21%) food isolates that have no predictive resistance determinants and were considered pan susceptible.

3.4.2 Source Type Food-Chicken

The majority (4634, 95%) of isolates from chicken were ST152; only 6 (<1%) chicken isolates were ST198, and none were Strain A. Of these 6 ST198 chicken isolates, 5 had no resistance determinants, and the other isolate was resistant to streptomycin, tetracycline, gentamicin, and hygromycin, but did not contain the QRDR mutations of interest and was not categorized as Strain A. Chicken isolates were most frequently resistant to streptomycin (4,369, 69%) and tetracycline (3,590, 57%) (Table 5).

3.4.3 Source Type Food-Other

There were 358 food isolates that were not categorized as chicken. Different food types included beef (n=95), pork (n=35), unidentified poultry (n=25), sesame seeds (n=8), meat mixtures (n=6), and alfalfa sprouts (n=1). There were 220 (70%) food isolates that were ST153, and 71 (23%) that were ST198. Of the 95 beef isolates, 41 (22%) were ST198 and 30 were ST152 (17%). Of the 71 isolates that were ST198, ten were characterized within Strain A and were taken from spices, seafood, or shellfish; all ten were resistant to ciprofloxacin (Table 5).

3.4.4 Environmental Isolates

There were 16 (<1%) isolates taken from environmental samples. Of these 16, eight (50%) were isolated from sources around chicken, one isolate was taken from cat food, one was taken from manure, and 6 (33%) isolates did not indicate a source of isolation. Eleven (69%) isolates were ST152 and five were ST198; all five that were ST198 did not indicate environmental source. Ten isolates had no resistance determinants and there were no environmental isolates characterized as Strain A or that had the QRDR mutations of interest.

3.4.5 Animal Isolates

There were 476 isolates taken from animal isolates. Of these, 459 (96%) were taken from cecal sampling, but did not indicate which animal. There were 285 isolates characterized as ST152 and 32 characterized as ST198. There were no animal isolates with the QRDR mutations of interest or characterized as Strain A. Refer to Table 5 for more information on antimicrobial resistance results for animal isolates.

Table 5. Antimicrobial Resistance of Source Type Food Chicken, Other Food, Animal, and Environmental Isolates

	Chicken N (%)[1]	Other Food N (%)[1]	Animal N (%)[1]	Environmental N (%)[1]
Amoxicillin-clavulanic acid	164 (4%)	10 (3%)	4 (1%)	0 (0%)
Ampicillin	246 (5%)	15(4%)	14 (3%)	0 (0%)
Azithromycin	0 (0%)	2 (1%)	0 (0%)	0 (0%)
Cefoxitin	221 (5%)	10 (3%)	12 (3%)	0 (0%)
Ceftiofur	58 (1%)	10 (3%)	12 (3%)	0 (0%)
Ceftriaxone	222 (5%)	10 (3%)	11 (3%)	0 (0%)
Chloramphenicol	1 (<1%)	1 (1%)	0 (0%)	0 (0%)
Ciprofloxacin[2]	26 (<1%)	10 (3%)	0 (0%)	0 (0%)
Colistin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gentamicin	100 (2%)	8 (2%)	6 (1%)	0 (0%)
Meropenem	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nalidixic acid	7 (<1%)	10 (8%)	0 (0%)	0 (0%)
Streptomycin	4,349 (71%)	228 (63%)	287 (60%)	6 (5%)
Sulfisoxazole	14 (<1%)	8 (3%)	0 (0%)	5 (4%)
Tetracycline	3,590 (58%)	219 (61%)	233 (48%)	0 (0%)
Trimethoprim-sulfamethoxazole	0 (0%)	2 (2%)	0 (0%)	0 (0%)
No predicted resistance	1,260 (20%)	171 (47%)	162 (34%)	10 (86%)
Resistance to ≥1 antimicrobial class[3]	2,017 (74%)	80 (65%)	314 (65%)	6 (5%)
Resistance to ≥3 antimicrobial classes[4]	51 (<1%)	8 (7%)	12 (3%)	0 (0%)
Total	6,167 (100%)	358 (100%)	476 (100%)	16 (100%)

1. Percents indicate column percent.
2. Ciprofloxacin resistance is defined as having one or mechanisms for resistance, or intermediate or ciprofloxacin non-susceptibility by AST.
3. Defined as being resistant to 1 or more antimicrobial agents in 1 or more antimicrobial classes.
4. Defined as having resistance to 3 or more antimicrobial agents in 3 or more antimicrobial classes.

CHAPTER 4: DISCUSSION

Results from this study support other findings that the MDR *Salmonella* Kentucky global strain is a public health concern. All 195 human isolates within Strain A were ciprofloxacin resistant and a majority were MDR. Furthermore, many Strain A isolates have resistance to other antibiotics that are used for treatment, including ampicillin (169, 86%), azithromycin (11, 5%), and ceftriaxone (18, 9%). Antimicrobial resistant *Salmonella* infections have been associated with poorer clinical outcomes, such as higher rates of hospitalization, longer hospital stays, and a higher risk of invasive infection (Parisi et al., 20160). Parisi et al. (2016) found that comorbidities are a potential risk factor for MDR infections since patients with comorbidities are more likely to be exposed to antimicrobial treatments and health care settings. There were 17 patients that reported underlying conditions. Furthermore, as MDR Kentucky is already resistant to several antibiotics, acquisition of additional resistant mechanisms could cause it to become extensively drug resistant and even harder to treat.

This study found that the majority of human cases of *Salmonella* Kentucky in the United States are Strain A, and a potential route of transmission for Strain A into the United States is imported food and international travelers. Results from this study support those of Tate et al. (2022). The overwhelming majority (84%) of *Salmonella* Kentucky from U.S. surveillance systems were food isolates from chicken. Despite this, most human isolates do not share characteristics with chicken isolates; most of the chicken isolates were ST152 while Strain A is ST198. Chicken isolates differed in resistance from Strains A as well. There is not enough information from this study to confidently determine the influence of imported foods on dissemination of this strain within the United States, but imported products were found to be contaminated with Strain A, including spices(n=5), snails (n=3), and shellfish (n=1). Source

information was not provided for these isolates. The products where Strain A has been found are not under routine surveillance and therefore Strain A may have gone undetected in similarly contaminated products.

A common exposure for patients with Strain A is international travel where 63% of those with travel information reported travel outside of the United States during the incubation period. In 2011, Hello et al., noted that 89% of travelers with ciprofloxacin non-susceptible *Salmonella* Kentucky infection traveled to northeastern and eastern Africa; however only 5 of 24 cases in this study reported travelling to this region. Other studies reported patients travelling to the same countries as those reported in this study; including Morocco, Tanzania, South Africa, and Indonesia (Vasquez et al.,2022; Bigell et al.,2022). In terms of potential reservoirs, Hello et al.,2011 noted that Egypt may be a reservoir of MDR *Salmonella* Kentucky, and other studies have concluded that as well.

Although Strain A did not share characteristics with chicken isolates from U.S. surveillance, another potential reservoir could potentially be chicken from other countries, specifically those that have reported this strain within their chicken supply. Countries that have report of the MDR Kentucky strain within their chicken production include Switzerland, Spain, and the United Kingdom (Vasquez et al 2021., Samper-Catieviela et al.,2022., Tasmin et al., 2017). Only one case reported eating chicken specifically while abroad. Eighteen of 21 (85%) of persons who had information available reported eating chicken in general, and 5 of those persons reported no travel. While this is a common exposure, it is difficult to hypothesize how significant these chicken exposures are in transmission of Strain A. FoodNet's population survey for 2018–2019 tool reports that 88% of American reported eating or having contact with chicken in the past 7 days, indicating that consuming chicken is a common exposure within the United State

4.1 Strain A vs. Other Salmonella Kentucky Human Strains

As mentioned previously, human isolates within Strain A do not share similar characteristics, like ST and antimicrobial resistance patterns, with *Salmonella* Kentucky isolates from chicken in the United States. Human isolates form Strain A also do not share similar characteristics with other human *Salmonella* Kentucky strains found within CDC surveillance systems. When comparing the difference in age distribution and geographic distribution of Strain A to other human Kentucky strains, there was a statistically significant difference. Strain A patients were more likely to be from the northeast and between the ages of 30–59, while other human Kentucky strains were more likely to be reported from the central United States and above the age of 60. Furthermore, while the majority of Strain A isolates were resistant to fluoroquinolones (195, 100%) and MDR (171, 87%), only four (3%) of other human strains were resistant to fluoroquinolones and 7(6%) were MDR. This furthers the hypothesis that Strain A may be coming from a different exposure than other human *Salmonella* Kentucky strains.

4.2 Strain A Surveillance

CDC's use of Strain A as a surveillance device to monitor the presence of the multidrug-resistant global strain described is successful and necessary. CDC monitors reoccurring, emerging, and persisting (REP) strains of concerning *Salmonella.* Similar to Strain A, these *Salmonella* strains may not cause acute outbreaks that warrant immediate investigation but may recur periodically or persist and cause illnesses over periods of month or years despite prevention efforts (REP Strain, 2023). Through routine surveillance of Strain A, valuable information for understanding the source and new reservoirs of Strain A has been captured.

CDC's use of PulseNet and Strain A has been successful at capturing the MDR global clone. Almost all Strain A isolates (99%) have the QRDR mutations of interest. There are 10 *Salmonella* Kentucky human isolates that do not meet Strain A's characterization requirements but do have ciprofloxacin resistance; of these 4 are ST198, 3 are ST152, 3 are ST314, and 1 is missing ST information. Three of ten isolates have the triple QRDR mutations of interest, 2 are ST198 and 1 is missing ST information. It can be hypothesized that the 3 ST198 isolates were not included because they are not genetically similar enough by cgMLST to the other Strain A isolates; *Salmonella* Kentucky is a polyphyletic serotype indicating that there may be greater strain diversity than other *Salmonella* serotypes. However, CDC's use of Strain A as a surveillance tool captured 98% of whole-genome-sequenced human isolates with the triple QRDR mutations of interest. There may be more isolates that were missed due to not being whole genome sequenced.

4.3 Impact from Covid-19 Pandemic

While not necessarily a limitation, the COVID-19 pandemic may also have impacted results to this study. During 2020, the overall incidence of *Salmonella* infection was significantly lower than the average annual incidence reported in 2017–2019 (Ray et al., 2021, Collins et al., 2022). Furthermore, it was noted that travel-associated illness was lower compared with other years (Ray et al., 2021). The COVID-19 pandemic impacted the number of people travelling to and from the United States as well as lab-capacity to sequence isolates. Between the years 2018–2019, there were 81 Strain A isolates uploaded to CDC surveillance systems; in 2020, there were 12. There was a 76% decrease in isolates uploaded to CDC surveillance system between the years 2019 and 2020; this is higher than the national reported drop in non-typhoidal *Salmonella* (serotypes not including *Salmonella* Typhi and *Salmonella* Paratyphi). This incremental decrease

is likely due to travel restrictions and decrease of travel observed in 2020. The effects of the pandemic cannot be fully assessed within this report, but it could have impacted the results.

4.4 Limitations

There are some limitations to using a REP strain approach for surveillance of the MDR *Salmonella* Kentucky global strain. First, to be identified within the strain, isolates must be whole genome sequenced and uploaded to PulseNet. As mentioned previously, many PulseNet participating laboratories may not have full sequencing capabilities and therefore not all isolates may make it into PulseNet. Second, the use of cgMLST for Strain A inclusion. Tate et al., 2022 documented two clades, a fluoroquinolone-susceptible clade (198.1) and a fluoroquinolone-resistant clade (Strain A, 198.2). cgMLST characterizes strains based on their core-genome and with *Salmonella* Kentucky as a serotype having greater gene-diversity documented, some isolates that may belong to clade 198.2 may be missed. There also may be isolates within CDC's surveillance system with PFGE data that are Strain A that will not be included because they may never undergo WGS. Third, whether Strain A is emerging within the United States cannot be assessed due to using WGS as a criterion in methodology. As mentioned previously, PulseNet participating laboratories did not begin fully using WGS for sequencing until 2019; therefore, it is difficult to determine whether the increase in cases leading up to 2019 is due to a true increase in incidence or increased uptake of WGS. Fourth, the variation in epidemiological information collection. CDC began requesting additional epidemiologic information from state health departments in 2020 for Strain A and therefore there is substantial missing exposure and travel information before the years of 2020–2022. While CDC does encourage public health partners to provide information, not all public health partners do; of 60 requests for case information sent, CDC received some epidemiological information for 42 cases. In addition, the epidemiological

information received was not standardized or complete and may be subject to reporter bias. A last limitation is ascribable to passive-isolate based surveillance systems like NARMS and PulseNet. While NARMS received WGS results from PulseNet, not all of the isolates were analyzed for resistance, and NARMS only received every 20th *Salmonella* isolate for AST. Over half of the isolates included in this analysis were not analyzed by NARMS; therefore, antimicrobial resistance analysis methods varied for isolates.

4.5 Future Considerations

In terms of continuing ongoing surveillance, CDC should continue to monitor Strain A. There was a significant increase in cases in 2019 before the 2020 pandemic, and after the pandemic, there was a slight increase of cases when comparing 2021 and 2022 data. By continuing to monitor, CDC can establish a 5-year baseline estimation of prevalence not affected by the pandemic for future surveillance purposes. They can also use travel information collected to assess potential international reservoirs.

Despite the recommendation that CDC should continue to monitor the strain, there are some recommended modifications for their ongoing surveillance. CDC has been performing enhanced surveillance to collect preliminary information on cases from this strain. This enhanced surveillance has been resource intensive and requires ample partner collaboration. While it has provided valuable information, given evidence that this strain is linked to international travelers, it may not be completely necessary. A suggestion for future surveillance of Strain A is to deploy enhanced surveillance techniques when a case suggests no travel, however, to collect basic travel, demographic, and clinical data from reporting states when a case is identified.

CHAPTER 5: CONCLUSION

MDR *Salmonella* Kentucky is an important public health concern in the United States. Persons experiencing MDR salmonellosis have been documented to have more severe health outcomes. If MDR *Salmonella* Kentucky were to acquire more resistance mechanism to other antibiotics, it could become extensively drug resistant and very difficult to treat. Evidence from this study supports other claims that MDR *Salmonella* Kentucky in the United States is related to international travel rather than domestic poultry. Furthermore, CDC's continued surveillance of MDR Kentucky is necessary to track the burden in the United States.

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