Antimicrobial Susceptibility in Washington State: A One Health Approach

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Abstract

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Antimicrobial resistance is rapidly increasing globally and is not only a human health concern but rather a One Health issue. Veterinarians and physicians have seen a rise in antimicrobial resistance in companion animals, food-production animals, and humans over the last few decades, with a paucity of new antimicrobials being developed. This cross-sectional study evaluated antimicrobial susceptibility testing from three sources: human National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) data from the CDC, retail meat NARMS data from the FDA, and clinical animal data from Washington Animal Disease Diagnostic Laboratory. The study had two purposes 1) evaluate the proportion of samples susceptible over time within each data source population in Washington and 2) characterize antimicrobial susceptibility in critical zoonotic pathogen genera (*Salmonella* and *Campylobacter*) in human, retail meat, and clinical animal samples using culture and

susceptibility data. The study found that antimicrobial susceptibility in human samples was significantly higher than in retail meat and clinical animal samples. However, human and retail meat sample populations showed a similar downward trend in susceptibility over time, whereas clinical animal sample susceptibility increased. Understanding what drove these changes in susceptibility is crucial to implementing effective policies, programs, and recommendations to reduce antimicrobial resistance. This research can serve as a baseline for monitoring antimicrobial susceptibility changes in Washington and guide future work to include general practice human and animal clinical samples along with environmental data to better represent antimicrobial susceptibility using a One Health approach.

Introduction

Antimicrobial resistance, a term used to describe drug resistance displayed by bacteria, fungi, parasites, and viruses, is a significant global One Health issue.[1] Recent estimates by the World Health Organization indicate that globally 700,000 deaths annually are due to drug-resistant infections and that if action is not taken, this number will climb to 10 million per year by 2050.[1] In the United States, 2.8 million people acquire a drug-resistant infection, and 35,000 of those cases succumb to the disease annually.[1] Although this growing problem has been a common topic in the last decade or two, little has been effectively done to slow its progression.

Antibiotic and antimicrobial are terms that are sometimes used interchangeably but have different definitions. An antibiotic is a medication that kills or inhibits the growth of bacteria, whereas antimicrobial is a broader term for a medication that kills or inhibits the growth of microorganisms, including bacteria, fungi, and viruses. Antibiotics and antimicrobials are broken up into different classes based on their chemical structure and characteristics, for example, their spectrum of activity or mechanism of action.

Antimicrobial resistance falls into two categories: intrinsic or acquired. Intrinsic resistance is present when bacteria are naturally resistant to a class of antibiotics.[2] For intrinsic resistance, the bacteria did not obtain their resistance through horizontal gene transfer or exposure to antibiotics.[2] Intrinsic resistance has been present for thousands of years, long before antibiotic use in human medicine, as bacteria present in glacier water from 2000 years ago were found to be resistant to ampicillin.[3]

When bacteria previously susceptible to a particular antibiotic show resistance to that antibiotic, it is called acquired resistance. Acquired resistance occurs when bacteria acquire

foreign DNA with resistance genes or through bacterial gene mutations.[3,4] Reducing acquired resistance is the primary focus of current antimicrobial resistance work.

Another level of complexity in antimicrobial resistance is the existence of multidrug-resistant (MDR) organisms. MDR organisms are typically defined as resistant to three or more different classes of antimicrobials.[5] Patients with MDR infections face increased mortality compared to individuals with infections caused by non-resistant bacteria, and the former cases result in a significant economic burden, with recent estimates in the United States exceeding 20 billion dollars annually.[4]

Many of the same antibiotics are used in humans and animals, which is a crucial reason why antimicrobial use and antimicrobial resistance patterns seen in each population are essential to monitor, as they may influence each other. Antimicrobial resistance continues to increase in human and animal populations due to multiple factors, including inappropriate use of antimicrobials; limited access to clean water and sanitation; lack of awareness of the issue; inadequate disease/infection prevention and control in medical facilities and on farms; limited access to appropriate treatments and diagnostics; and insufficient enforcement of regulations.[1] As the world becomes more globalized, microbes are regularly exposed to new locations, hosts, and resistance genes worldwide, meaning that the above factors now have global implications.[3] Steps can and are being taken to manage resistance, including combination therapy (use of multiple antibiotics), implementation of antimicrobial stewardship programs, use of infection control and prevention programs, and exploring alternative therapies, including phages, topical agents, and immune stimulation.[3]

These efforts are essential in decreasing the pace of progression or potentially reducing antimicrobial resistance; however, another factor already impacting our ability to treat patients with resistant infections is the paucity of new antimicrobials. Starting in the 1990s, the discovery of new antimicrobial agents has declined steeply.[5] Currently, new drugs are unable to keep pace with the continued emergence of resistance genes, with only 15 new antibiotics approved between 2000-2018.[3,6] Production of new antibiotics is limited due to poor return on investment for pharmaceutical companies because of the paradoxical reality that new antimicrobials will be judiciously used to avoid increasing selective pressure for resistance.[3]

Antimicrobial resistance is not only a human health concern but rather a One Health issue. One Health is the collaborative transdisciplinary approach to ensure the health of humans, domestic animals, wildlife, plants, and the environment.[7] Veterinarians and physicians have seen a rise in antimicrobial resistance in companion animals, food production animals, and humans over the last few decades.[4,8] A One Health approach to solving this growing problem will allow us to tackle antimicrobial resistance from multiple directions and at many levels. This will increase our chance of improving the health of all populations as a decrease in resistance in one population or species will likely have positive effects on slowing resistance in another as many pathogens are zoonotic, and acquired resistance can be shared among bacteria.

Unfortunately, there is no single database to store comprehensive data on antimicrobial resistance at the state or national level in the United States. The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) is the largest U.S. antimicrobial database and includes data from the U.S. Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA) along with state health departments and institutions of higher education.[9] NARMS monitors

antimicrobial resistance in foodborne bacteria and other intestinal bacteria from humans, retail meat, and livestock animals (cecal samples).[9] This database has a narrow focus and generally does not include non-foodborne or non-enteric bacteria and excludes information regarding antimicrobial resistance in plant agriculture and the environment. In 2017 and 2018 (respectively), the NARMS database expanded to include antimicrobial susceptibility data on companion animals (dogs) for *E. coli* and *S. pseudintermedius* from the FDA's Veterinary Laboratory Investigation Response Network and USDA's National Animal Health Laboratory Network.[9,10] The lack of a comprehensive database makes evaluating the extent of antimicrobial resistance in the United States challenging and is a common issue globally.

A recent French study found that resistance trends in humans compared with companion and food animals were statistically different and concluded that resistance dynamics might be species-specific due to differing antibiotic practices.[8] Although the general assumption is that antimicrobial resistance in microbes cultured from humans and animals is related, few studies have analyzed or compared data between these populations.[8] To see if trends in resistance are similar between humans and animals and if they could predict broader increases in resistance, overall studies directly comparing human and animal isolates are needed.

This study compiled and reviewed human and animal antimicrobial data for the state of Washington. The data was used to update the Washington Integrated Surveillance for Antibiotic Resistance (WISAR) database to provide a more current antimicrobial resistance picture for Washington state. Data were collected from human NARMS data from the CDC, retail meat NARMS data from the FDA, and clinical animal data from Washington Animal Disease Diagnostic Laboratory (WADDL).

There were two aims of this study. The first was to evaluate the proportion of samples susceptible over time within each data source population in Washington. The second was to characterize antimicrobial susceptibility in critical zoonotic pathogen genera (*Salmonella* and *Campylobacter*) in human, retail meat, and clinical animal samples using culture and susceptibility data.

Methods

STUDY DESIGN

This was a descriptive cross-sectional study. Culture and susceptibility data used to create the dataset evaluated in this study came from humans (NARMS), retail meat (NARMS), and living animals (WADDL) within the state of Washington from 2002 through 2022.

DATA SOURCES AND ELIGIBILITY CRITERIA

Human data from 2002 through 2022 was obtained from the CDC NARMS laboratory, which performs culture and susceptibility testing on bacterial surveillance samples from public health laboratories and outbreaks investigations of illness in humans.[11] Routine surveillance samples are submitted to the CDC NARMS laboratory from public health laboratories. [11] These laboratories submit every 20th non-typhoidal *Salmonella, Shigella, and Escherichia coli* O157 sample and all Salmonella serotype Typhi, serotype Paratyphi A, serotype Paratyphi C and *Vibrio (*other than *V. cholerae)* isolates they receive. [11] The public health laboratories from state health departments that participate in the CDC's Foodborne Diseases Active Surveillance Network (FoodNet) will forward some *Campylobacter* isolates for testing at the CDC NARMS laboratory.

[11] The human data collected and analyzed included the following enteric bacteria: *Salmonella spp., Campylobacter spp., Escherichia coli O157, Vibrio spp.,* and *Shigella spp*.[12]

The FDA collects retail meat samples from grocery stores within selected states for the NARMS database. Collected samples are tested for susceptibility to multiple antimicrobials. Specific retail meat samples collected from grocery stores include chicken, ground turkey, ground beef, pork, shrimp, tilapia, and salmon.[12] However, samples are currently only collected in 25 states and territories, and within Washington, were only collected for chicken, ground turkey, ground beef, and pork.[13] Retail meat for the NARMS database is tested for *Salmonella spp., Campylobacter spp., Escherichia coli, Enterococcus spp., Vibrio spp.,* and *Aeromonas spp.* For this study, data available for Washington state covered 2012 through 2019 and only included *Salmonella spp.* and *Campylobacter spp*.

This dataset's clinical animal culture and susceptibility data were obtained from WADDL from 2002 through 2022. Samples were received from companion animals, production animals, and wildlife from various sources across the state, including the Washington State University Veterinary Teaching Hospital, corporate and privately owned veterinary clinics, livestock facilities, and others. A wide variety of bacterial organisms were cultured as data were clinically focused and not solely concentrated on zoonotic pathogens, as in the NARMS data.

Samples were included in the study if they had positive bacterial growth on culture with an identifiable organism. Negative culture results were excluded from the analysis. Each sample was viewed as a "new" or unique sample, with the exception of the samples that had undergone Whole Genome Sequencing and were entered twice. Otherwise, duplicate, or multiple samples from the same sample source, if present, were not removed from the data because they could not

be identified, or samples were taken from separate collection points (e.g., skin, urine) or on different dates (e.g., repeat AST to monitor treatment).

DATA COMPILATION

Data cleaning was a time-intensive process for this project as there were five separate datasets (one NARMS human, one NARMS retail meat, and three WADDL) that had to be prepared for combining into a final dataset. Although human and retail meat data were from the NARMS database, the formatting, variables, and naming structures varied. WADDL had multiple software changes over the period evaluated. This led to data being reported in numerous file types and significant variations in variables, naming structure, and source details available.

The data used to create this dataset were collected for different purposes: antimicrobial surveillance (NARMS retail meat), surveillance and outbreak investigation (NARMS human), and clinical care (WADDL animal). It is important to note that none of the data were collected specifically for this study.

VARIABLES

The following variables were included in the compiled dataset: genus (of bacterium), species (of bacterium), month of collection, year of collection, host species (species sample was collected from), data source (NARMS animal meat, NARMS human, WADDL animal clinical), and susceptibility to ten antibiotics. Commonality among the three data sources was important when determining what bacterial organisms and antibiotics to analyze. Given the wide variety of bacterial species present in each genus of bacteria, data were analyzed at the genus level. *Salmonella spp*. and *Campylobacter spp*. were common to all three data sources.

Table 1 lists the classes of antimicrobials and associated antimicrobial drugs included in the compiled dataset as they were in each original dataset. These antimicrobial classes were based on those defined by the Clinical and Laboratory Standards Institute (CLSI) and had been used for the NARMS data.[14]

Table 1.CLSI Antimicrobial Classification

Antimicrobial Class	Antimicrobial Drug
Aminoglycosides	Gentamicin
Beta-lactam/Beta-lactamase Inhibitor Combinations	Amoxicillin-Clavulanic Acid
Folate Pathway Inhibitors/Antagonists	Trimthoprim-Sulfamethoxazole
Macrolides	Azithromycin, Erythromycin
Penicillins	Ampicillin
Phenicols	Chloramphenicol, Florfenicol
Tetracyclines	Tetracycline
Lincosamides	Clindamycin

When reviewing antimicrobial susceptibility testing (AST) results, it is imperative to understand a few key terms: Minimum inhibitory concentration (MIC), breakpoint, and susceptibility categories. The MIC is the measured concentration of an antimicrobial agent required to kill or inhibit the growth of a bacterium or fungi and is used to define breakpoints.[15] There are multiple breakpoint types referenced in AST. A microbiological breakpoint uses the

MIC for a specific antimicrobial that separates wild-type bacterial populations from populations with acquired or selected resistance (in vitro testing).[16] This may also be referred to as a wild-type breakpoint or epidemiological (wild-type) cutoff value.[16] A clinical breakpoint differentiates bacterial strains with a good chance of treatment success from those where the antimicrobial is likely to be unsuccessful in treating the infection.[16] A pharmacokinetic (PK) /pharmacodynamic(PD) breakpoint is used to describe antimicrobial concentrations that are calculated based on the PK/PD parameters of a drug within an animal model (in vivo) and are then adjusted to apply to humans.[16] Understanding which breakpoint is being used and reported is important when evaluating AST results.

Susceptibility categories are defined by breakpoints specific to each microbial organism and antimicrobial agent pairing. These have typically been reported as susceptible "S," intermediate "I," and resistant "R." Under these categories, it has been common for "I" and "R" to be grouped together by microbiologists, clinicians, and regulatory agencies, which treated "I" as resistant (or non-susceptible), affecting therapeutic decisions.[15] The European Committee of Antimicrobial Susceptibility Testing (EUCAST) redefined the definitions for the susceptibility categories in 2020 while retaining the acronyms of "S," "I," and "R."[15] "S" now indicates an organism is susceptible at a standard dosing regimen, "I" susceptible with increased exposure, and "R" indicates a high likelihood of treatment failure even with increased exposure to an antimicrobial.[17] (Table 2)

Table 2. EUCAST Susceptibility Level Recommendations

S - Susceptible, standard dosing regimen	A microorganism is categorized as this when there is a high likelihood of therapeutic success using a standard dosing schedule for the antimicrobial agent
I - Susceptible, increased exposure	A microorganism is categorized as this when there is a high likelihood of therapeutic success because exposure to the antimicrobial is increased by adjusting the dosing schedule or by its concentration at the site of infection
R - Resistant	A microorganism is categorized as this when there is a high likelihood of therapeutic failure even with increased exposure to the antimicrobial

Bacterial organisms in this dataset were reported as being susceptible or resistant to each antimicrobial they were tested against based on laboratory-assigned MICs and breakpoints. To evaluate susceptibility within the data, a bacterium sample was counted as susceptible if it was not resistant to any antibiotics it was challenged against. It was viewed as resistant if it was resistant to at least one antibiotic.

Breakpoints provided for the human and retail meat NARMS data were used to determine the presence or lack of resistance for each sample to a particular antimicrobial agent. Breakpoints were generally based on the CLSI values and were common between the NARMS retail meat and NARMS human data. Breakpoints for the WADDL data were not available for all samples, but the resistance classification of “S,” “I,” or “R” that was reported to the veterinarian that

submitted the sample was available. The susceptibility classification of "S," "I," and "R" were used to compare the data. Not all bacterium/antimicrobial combinations had an option for an "I" value. For this reason and the ease of evaluating susceptibility, the three levels were condensed into susceptible and resistant. "S" and "I" levels were combined into a single level of susceptibility based on EUCAST recommendations.[15]

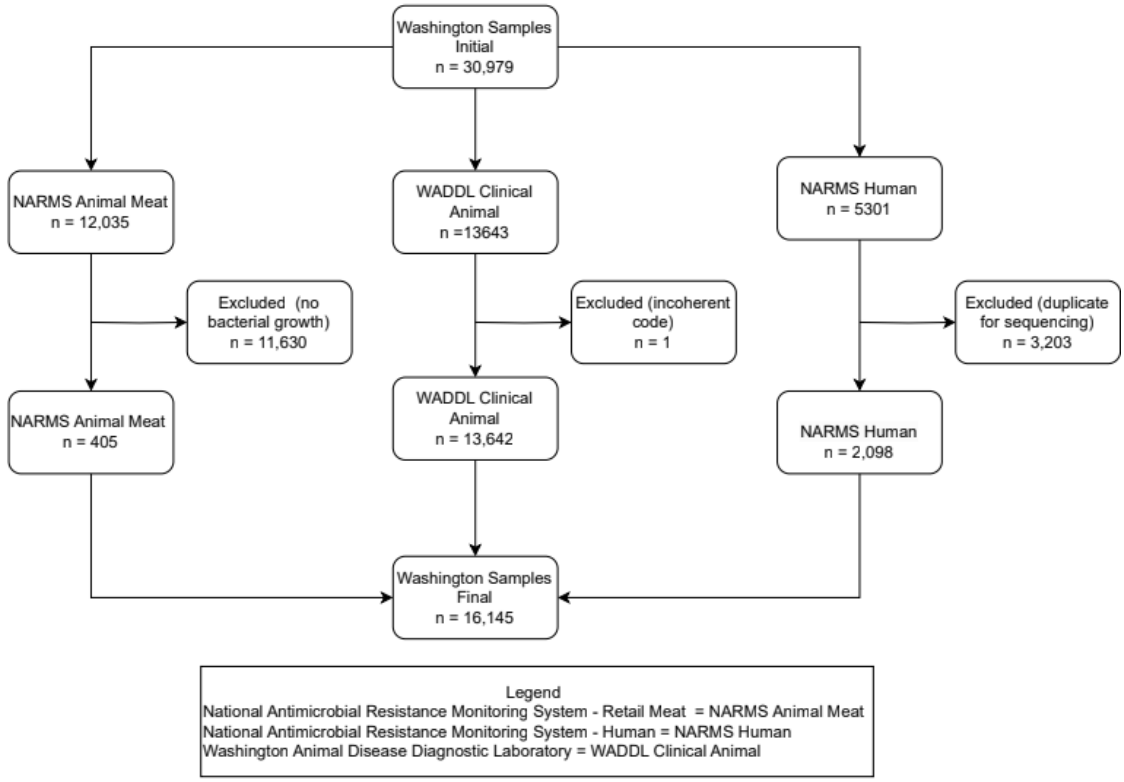
DATA ANALYSIS

Statistical analysis was performed using R Software V4.1.1.[19] A population proportion was calculated to determine the percentage of bacteria within each data source population (NARMS Human, NARMS Animal Meat, WADDL Animal Clinical) susceptible to antimicrobials for a given year. A binomial proportion confidence interval was then calculated for each population proportion at a confidence level of 95%.

Results

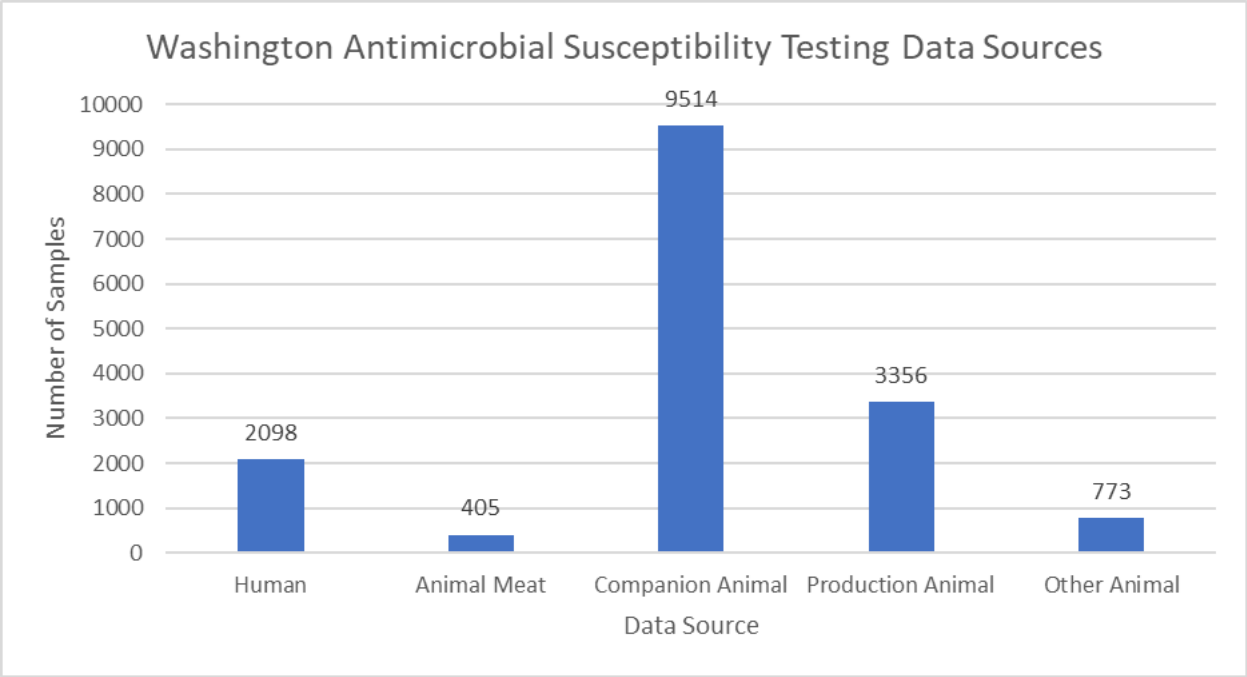
Antimicrobial resistance data from Washington were collected from three sources, each with a different population type, humans (NARMS human), animal meat (NARMS retail meat), and clinical animal (WADDL). Initial data consisted of 30,979 data entries, but after excluding samples with no bacterial growth, duplicate samples for Whole Genome Sequencing (WGS), and those that were incoherent, the final dataset included 16,145 samples. In the last dataset, human samples contributed ~13%, animal meat <1%, and clinical animals ~86% to the total number of samples. (Figure 1).

Figure 1. Summary of Source Samples



A bar graph illustrating the distribution of antimicrobial samples for Washington is depicted in Figure 2. Data were split into five groups: human (NARMS human data), animal meat (NARMS retail meat), and animal clinical data from WADDL were divided into three groups: companion animal, production animal, and other animal. WADDL clinical animal data were broken into three categories to differentiate expected human-animal exposures, with companion animals having frequent exposure, production animals having limited exposure, and other animals having rare exposure to humans.

Figure 2. Antimicrobial Susceptibility Testing Data Sources

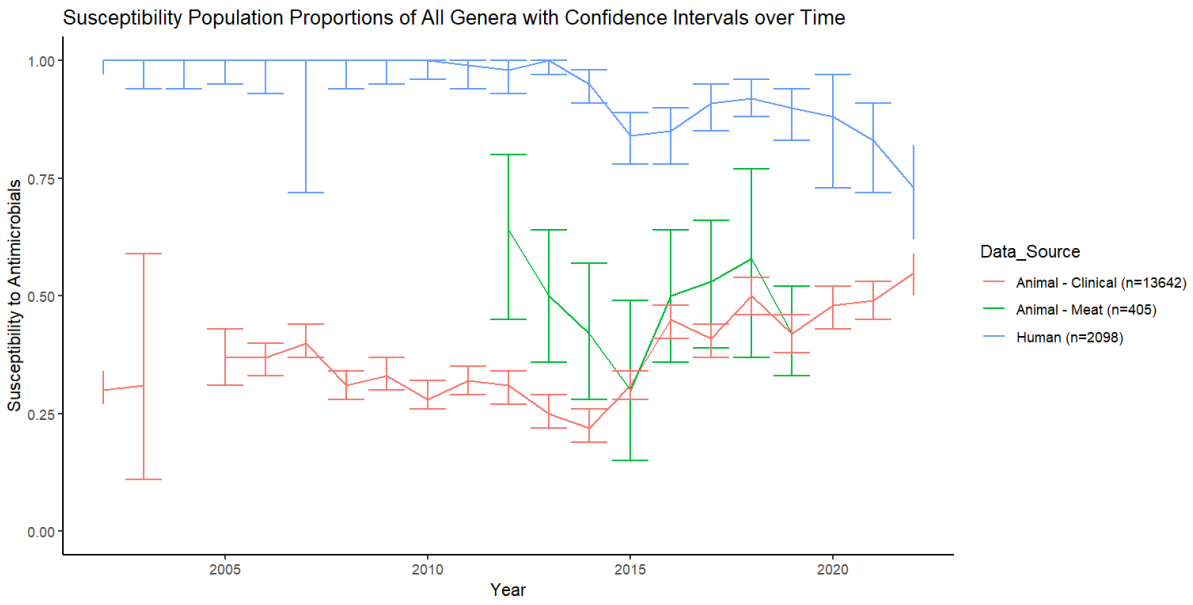


SUSCEPTILITY OF ALL GENERA

Figures 3 and 4 provide an overview of the change in susceptibility of all bacteria in each population group over time. Although using the same data, these figures present a different perspective to the reader. Figure 3 presents the data with confidence intervals, and Figure 4 uses data smoothing and a trend line for ease of reading.

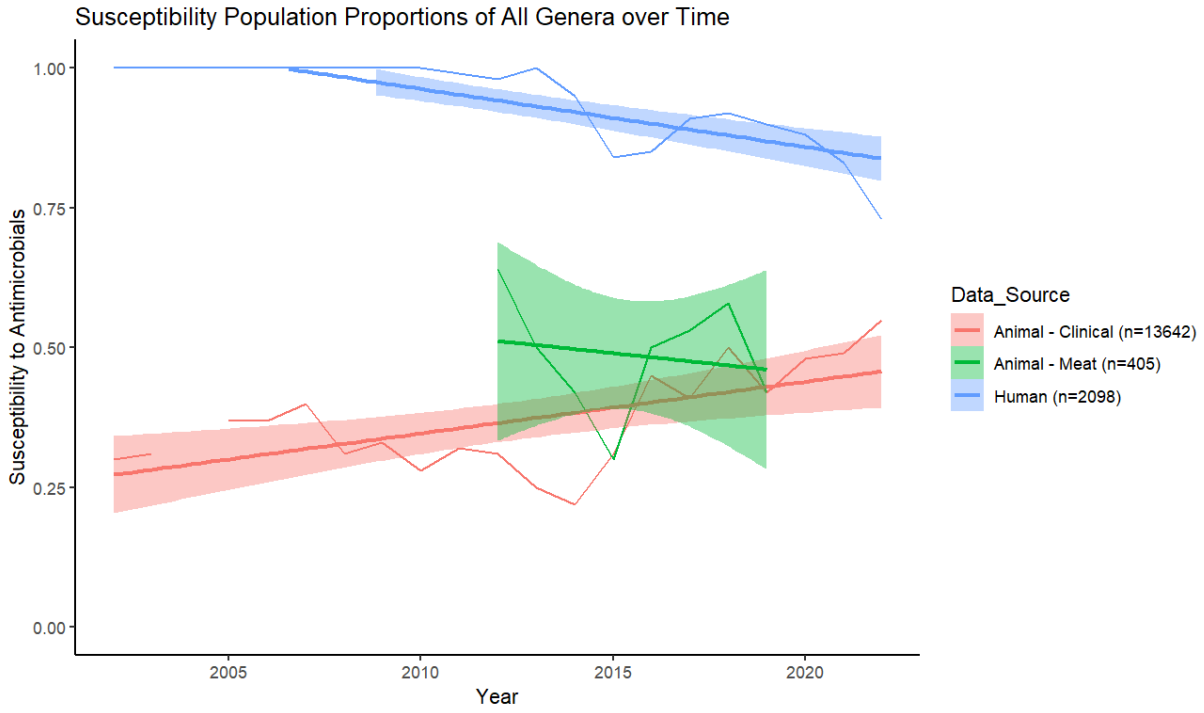
Population proportions of susceptibility and confidence intervals were calculated yearly for all samples within each source population (human, animal meat, clinical animal) and graphed. (Figure 3). A bacterium sample was counted as susceptible if it was not resistant to any antibiotics it was challenged against and was viewed as resistant if it was resistant to at least one antibiotic.

Figure 3. Susceptibility Population Proportions of All Genera Samples by Source Type with Confidence Intervals



A trend line was added and data smoothing of the population proportions and confidence intervals was performed in Figure 4 to illustrate the trend over time in each population clearly. Susceptibility within human and animal meat decreased, and clinical animal susceptibility increased.

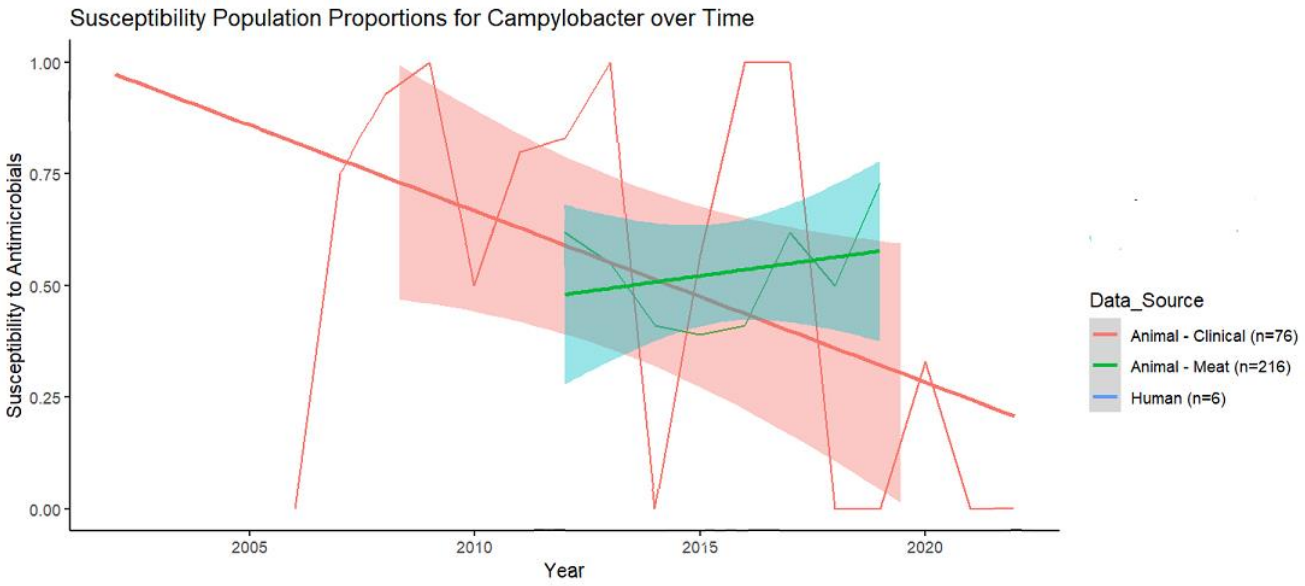
Figure 4. Susceptibility Population Proportions of All Genera Samples by Source Type (Smoothed with Trend Line)



SUSCEPTILITY OF CAMPYLOBACTER SPP.

The data was further broken down to evaluate susceptibility over time in *Campylobacter spp*. samples as this genus was present in all three source populations. Due to meager sample numbers in humans (only six samples were found in a single year), only animal meat and clinical animal data were evaluated. In Figure 5 *Campylobacter spp.* in animal meat showed generally consistent susceptibility over time. In contrast, susceptibility varied considerably in clinical animals each year, and the confidence intervals were wide due to the low sample size. Clinical animal AST results indicated that most samples were susceptible in the early 2000s, but in recent years (with only two to three samples per year), very few were susceptible.

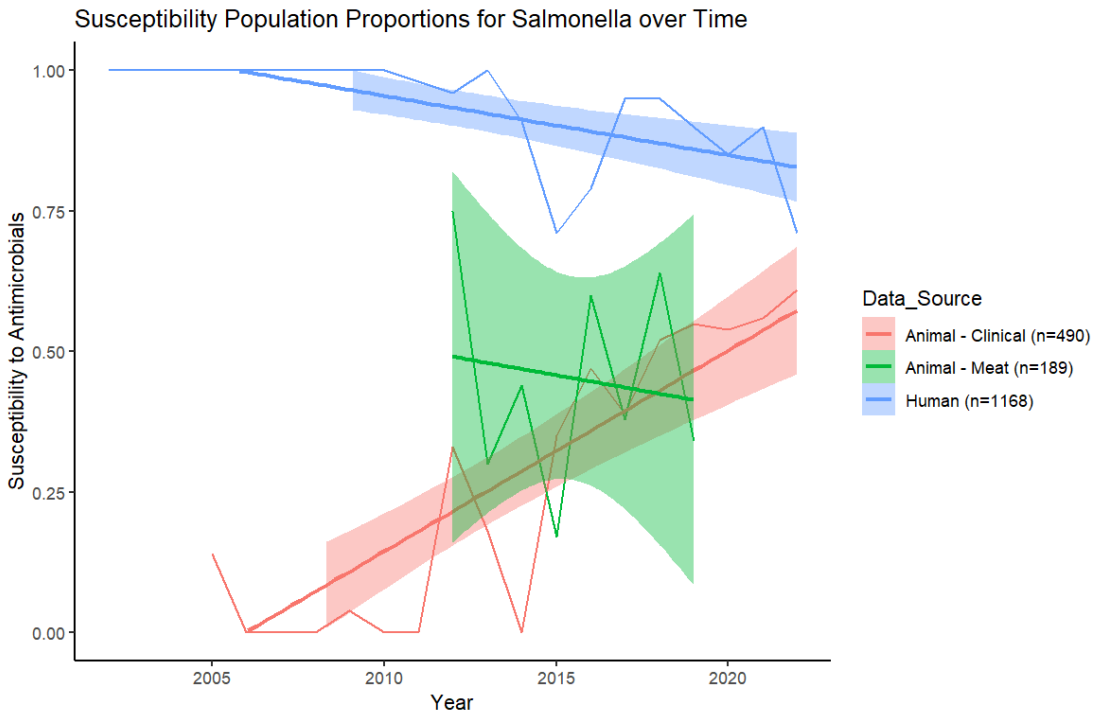
Figure 5. Susceptibility Population Proportions of Campylobacter spp. by Source Type



SUSCEPTILITY OF SALMONELLA SPP.

Susceptibility of *Salmonella spp*. to chloramphenicol, erythromycin, amoxicillin-clavulanic acid, ampicillin, clindamycin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole was evaluated over time as it was common to all source populations. A combined graph with a trend line and data smoothing showed decreasing susceptibility in humans and animal meat and increasing susceptibility in clinical animal data. (Figure 6).

Figure 6. Susceptibility Population Proportions of Salmonella spp. by Source Type



Clinical animal data showed low susceptibility in the early 2000s, with a steady susceptibility increase starting in 2015. Sample size each year varied a fair amount, with approximately 30% of the years having less than ten samples and the remaining with 20-60 samples, which explains the variation in confidence intervals. Human *Salmonella* cases saw their first resistant sample in 2011. Over time susceptibility has decreased and given the larger sample sizes with tighter confidence intervals is more likely to represent actual change. Animal meat averaged around 18 *Salmonella* samples annually until 2019, so confidence intervals were wide. *Salmonella* susceptibility in animal meat was significantly lower than for humans, but a gentle downward trend was similar in both groups. Alternatively, *Salmonella* susceptibility in clinical animal AST results increased over time.

Discussion

This analysis of antimicrobial susceptibility in Washington complements prior work completed by WISAR and provides a picture of antimicrobial susceptibility changes over time within the state. Limited work has evaluated human, retail meat, and clinical animal antimicrobial susceptibility data, with no known studies with this broad focus conducted in Washington. Historical susceptibility studies that include human, production animal, and retail meat AST have focused on a specific organism (e.g., *E. coli*), typically in a local area. This analysis sought to provide a broader view of susceptibility across a region.

Each sample in this study was viewed as a "new" or unique sample. It is possible that in rare cases, multiple samples may have been submitted for the same human or animal patient (e.g., feces, respiratory secretions) but were viewed as individual samples as the bacteria grown and susceptibility results were expected to be distinct given source differences or time intervals between sample collections. It was unlikely that multiple samples of the same source (e.g., urine, skin) from the same patient would be submitted on the same day, so concern about duplicate samples was relatively low.

Four assumptions were made when choosing to use the binomial test. The first was that the samples were dichotomous. We consolidated the three outcomes of "S" (susceptible), "I" (intermediate), and "R" (resistant) into two categories. A result of "R" remained unchanged, but "S" and "I" were grouped into a single result of "S." The second assumption was that the sample size being evaluated was significantly less than the population size. This was easily met as the data was only a small sample of culture and susceptibility samples performed in Washington. Next, it was assumed that the sample represented the population fairly. For this analysis, we

accepted this assumption but will discuss later the limitations of this assumption. Finally, we assumed the samples were independent of each other. It is possible that a patient had multiple samples within the data, but for this analysis, we assumed this would be quite rare and that the source of the sample (e.g., skin, abscess, respiratory secretions) and organism(s) cultured and or susceptibility of the organism(s) would not have a significant impact on the findings.

Human AST results, which included six bacterial genera, had the greatest susceptibility to antimicrobials (least resistance) within the three data sources. Susceptibility in this group appears to have slowly declined since 2010; however, overall susceptibility within human AST each year was significantly higher than animal meat or clinical animal AST. Animal meat AST demonstrated a similar downward trend in susceptibility to the human data, but susceptibility overall was much lower. These two populations (human and animal meat) used the same MICs and breakpoints for shared organisms, allowing them to be directly compared. The similar downward trend observed in these populations likely represents an actual negative change in susceptibility within these populations.

Clinical animal AST revealed low susceptibility to antimicrobials that, over time, improved. Other studies have seen improved susceptibility of bacteria after altering local and state antimicrobial practices. A study in dairy calves in Washington found *E. coli* cultured from calves in groups that received multiple antimicrobial treatments were more likely to have extensive resistance (low susceptibility) than those from groups with minimal antimicrobial treatments.[18] In California, antimicrobial resistance in human urine decreased after a new senate bill restricted specific antimicrobial practices (routine preventative use or use without veterinary prescription) in production animals within the state.[20] Although superficially, the increased

susceptibility in clinical animal samples appears encouraging, it may or may not represent a real phenomenon, as a clear cause for this change is not apparent.

Comparing animal data between veterinary laboratories presents challenges as some may use clinical breakpoints and others epidemiological cutoff values.[21] Even within a given laboratory, breakpoints or methods used for testing may have changed over time, which adds difficulty in comparing results within the same program.[21] Standardization is lacking in this area of veterinary medicine.

In this dataset, the confidence intervals for all animal meat samples were quite broad, and in general, the clinical animal data had the narrowest confidence intervals. Sample size within each given year is a key contributor to these populations' variation in confidence intervals. There were significantly more clinical animal AST samples than human or animal meat. In an ideal situation, this would indicate that the AST changes over time for clinical animals closely represents what is being seen in live animals.

Understanding how AST results are grouped is essential when reviewing changes in susceptibility. Until 2020 when EUCAST updated their recommendations for “S” and “I” to be grouped as susceptible, almost all previous research grouped “I and “R” together as resistant. This makes it more challenging to compare antimicrobial susceptibility papers and data prior to 2020 to the present. Current research may follow either way of grouping data, so it requires a careful review before directly comparing findings, as how data is grouped may significantly alter the susceptibility reports generated. This study followed the EUCAST recommendations for grouping “S” and “I” together as susceptible.

Campylobacter spp. and *Salmonella spp.* were selected to explore susceptibility further as the only two genera present in all populations. *Campylobacter spp.* in animal meat showed generally consistent susceptibility over time but varied considerably in clinical animals each year, with a large drop in susceptibility in the last few years. The low sample size and sudden reversal in susceptibility make it challenging to determine what change, if any, may be present. It could be that since WADDL likely receives more samples from cases that have failed prior therapy, the recent samples were genuinely resistant, or they may be a poor representation of *Campylobacter spp*. Either way, no meaningful interpretation can be made with this data.

Similar to humans, treating *Salmonella* infections in animals with antibiotics is uncommon, and testing at WADDL is performed almost solely for antimicrobial resistance surveillance. *Salmonella* susceptibility results from WADDL are rarely reported to veterinarians as there are no appropriate interpretations (e.g., no breakpoints for cattle, the primary population *Salmonella* is seen in), and antimicrobial treatment with certain drugs in agricultural animals is controversial or illegal.[24]

Using antimicrobials or other medications in production animals (animals raised for food) poses a potential risk of harmful drug residues being present in tissue that is consumed by humans.[25] To limit this risk, medications used in livestock have drug labels that define how and when a drug may be used. The label provides guidance on conditions it may be used for, dosage and route of administration, what species may be given the medication, and the withdrawal time after treatment. In veterinary medicine, a withdrawal time (period) defines the amount of time required for an animal to metabolize a medication and the amount of time needed for the concentration level of that drug to decrease to an acceptable level in tissue or milk.[25] In some

cases, a drug may be prohibited for use in certain animal species due to a potential risk to humans of causing cancer, toxic reactions, or antimicrobial resistance.[25]

Out of the three populations evaluated in this study, overall susceptibility in WADDL AST samples was generally low but appeared to improve steadily over time. MICs and breakpoints for some organisms changed throughout data collection, but it is not clear whether they were better or more appropriate. The approach to breakpoint application was adjusted as new research was released and laboratory leadership transitioned. Plates used for culture and susceptibility testing have seen limited changes in the last few decades. Unfortunately, few improved antibiotics were released, so they were not likely associated with the changes seen. Discerning whether the change in overall susceptibility in WADDL data is real proves challenging.

LIMITATIONS

A key limitation of this study is that the findings in this analysis may not represent antimicrobial susceptibility across the state. The dataset evaluated was missing human general practice clinical data, animal general practice clinical data, and environmental data. Including these would more fully encompass a One Health approach and provide a more comprehensive view of antimicrobial susceptibility change in Washington.

Each data source used in this study presented unique limitations. WADDL AST results may not appropriately represent clinical animal data within the state as routine AST performed in general practice veterinary clinics are typically submitted to laboratories such as Antech, Idexx, and Zoetis, which were not included. Given its association with the Washington State University Veterinary Teaching Hospital and its role as the animal disease diagnostic laboratory for the state,

it likely received an overrepresentation of treatment failure and complicated cases. These cases may be more likely to have resistant organisms than an average clinical case in Washington and may underestimate antimicrobial susceptibility. Clinical animal samples submitted may have only represented cases that veterinarians deemed more severe or had been performed on patient samples from clients with the financial means to pay for testing. Each of these could skew the WADDL AST result population.

Data from each NARMS source focused solely on zoonotic organisms, whereas WADDL AST results included both zoonotic and non-zoonotic bacteria in this dataset. This means that changes in susceptibility within the overall WADDL data are not directly relatable to human risk, and the presence or lack of susceptibility changes seen in one genus or host species may not indicate future antimicrobial resistance in another. Further comparisons focused solely on AST results from zoonotic bacteria could be made by subsetting the WADDL data.

Unfortunately, it is not appropriate to compare human or animal meat AST directly to clinical animal AST as there are significant differences in bacteria screened, breakpoint determinations, and interpretations.[21] In animals, the use of different terminology, techniques, and clinical breakpoints makes it challenging to compare them to human data.[21] These differences are due to variations in host species factors such as susceptibility to a bacterium, pharmacokinetic and pharmacodynamic considerations, and the ability to safely or legally use a specific medication within a particular animal species. Additionally, MICs and breakpoints for specific bacteria-drug combinations are frequently unknown in animals due to a lack of available data. This leads laboratories to use human MICs and breakpoints for animals or extrapolate known values from one species, for example, cattle to sheep and goats, because they are also

ruminants.[22] However, assuming that breakpoints approved for one animal species will be the same for another species is inappropriate, so it must be done cautiously.[23]

Animal meat was only tested for two genera of bacteria, compared to six for humans and over 80 for clinical animals, providing a more limited view of susceptibility. The animal meat AST results only represent what Washingtonians were exposed to rather than reflect bacterial population susceptibility in production animals in the state. Retail meat sold in Washington originates from production animals across the United States. This is a crucial point, as changes in antimicrobial susceptibility seen in this data are affected by production practices within and outside Washington. The small AST sample size in animal meat is not ideal for this study, but overall is a positive finding for public health, as only 3.4% of meat samples collected in Washington were positive for bacterial growth (*Campylobacter spp.* and *Salmonella spp.*), indicating a safe food source.

Conclusion

Antimicrobial susceptibility in Washington appears to be slowly declining in human and animal meat at a similar rate and may be improving in clinical animals. The latter finding is interesting and requires further investigation as antimicrobial susceptibility in human, animal, and environmental populations is generally declining globally. Understanding what drives these changes in susceptibility is crucial to implementing effective policies and programs to reduce antimicrobial resistance. Recent policy changes on antimicrobial use in production animals in California have seen early positive results and may indicate that similar changes in other states may reap similar benefits.[20]

In trying to evaluate susceptibility changes over time, this study identified multiple reasons evaluating data across multiple population sources is challenging, along with areas that require further research. This work provides insight into antimicrobial susceptibility changes in Washington across multiple sources and species over time and will hopefully serve as a baseline for monitoring future changes in susceptibility within the state.

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Appendix A: Supplementary Data

Table 3. Susceptibility Population Proportions and Confidence Intervals Human Samples

Human Samples						
Year	Susceptible	Resistant	Total	Population Proportion (Susceptible)	Lower CI	Upper CI
2002	139		139	1	0.97	1
2003	59		59	1	0.94	1
2004	58		58	1	0.94	1
2005	68		68	1	0.95	1
2006	54		54	1	0.93	1
2007	11		11	1	0.72	1
2008	56		56	1	0.94	1
2009	77		77	1	0.95	1
2010	96		96	1	0.96	1
2011	94	1	95	0.99	0.94	1
2012	97	2	99	0.98	0.93	1
2013	140	0	140	1	0.97	1
2014	143	7	150	0.95	0.91	0.98

2015	150	28	178	0.84	0.78	0.89
2016	121	22	143	0.85	0.78	0.9
2017	122	12	134	0.91	0.85	0.95
2018	209	17	226	0.92	0.88	0.96
2019	123	14	137	0.9	0.83	0.94
2020	30	4	34	0.88	0.73	0.97
2021	55	11	66	0.83	0.72	0.91
2022	57	21	78	0.73	0.62	0.82

Table 4. Susceptibility Population Proportions and Confidence Intervals Animal Meat Samples

Animal Meat Samples						
Year	Susceptible	Resistant	Total	Population Proportion (Susceptible)	Lower CI	Upper CI
2012	21	12	33	0.64	0.45	0.8
2013	26	26	52	0.5	0.36	0.64
2014	21	29	50	0.42	0.28	0.57
2015	9	21	30	0.3	0.15	0.49
2016	26	26	52	0.5	0.36	0.64
2017	29	26	55	0.53	0.39	0.66

2018	15	11	26	0.58	0.37	0.77
2019	45	62	107	0.42	0.33	0.52

Table 5. Susceptibility Population Proportions and Confidence Intervals Animal Clinical Samples

Animal Clinical Samples						
Year	Susceptible	Resistant	Total	Population Proportion (Susceptible)	Lower CI	Upper CI
2002	198	608	806	0.25	0.22	0.28
2003	5	11	16	0.31	0.11	0.59
2004			0			
2005	69	203	272	0.25	0.2	0.31
2006	182	588	770	0.24	0.21	0.27
2007	268	667	935	0.29	0.26	0.32
2008	217	754	971	0.22	0.2	0.25
2009	207	689	896	0.23	0.2	0.26
2010	203	724	927	0.22	0.19	0.25
2011	220	730	950	0.23	0.21	0.26
2012	176	578	754	0.23	0.2	0.27

2013	144	567	711	0.2	0.17	0.23
2014	111	535	646	0.17	0.14	0.2
2015	209	635	844	0.25	0.22	0.28
2016	291	441	732	0.4	0.36	0.43
2017	226	428	654	0.35	0.31	0.38
2018	269	325	594	0.45	0.41	0.49
2019	211	373	584	0.36	0.32	0.4
2020	175	328	503	0.35	0.31	0.39
2021	229	390	619	0.37	0.33	0.41
2022	215	243	458	0.47	0.42	0.52

Figure 11. Susceptibility of All Samples by Source Type (Smoothed with Trend Line) (S vs RI)

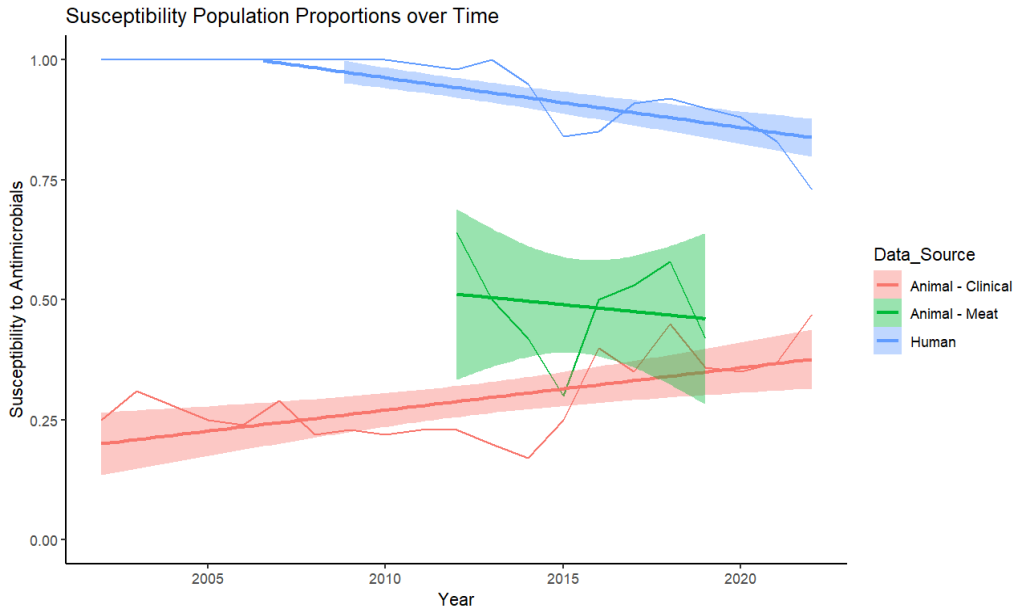

Figure 12. Susceptibility of All Samples by Source Type with Confidence Intervals (S vs RI)

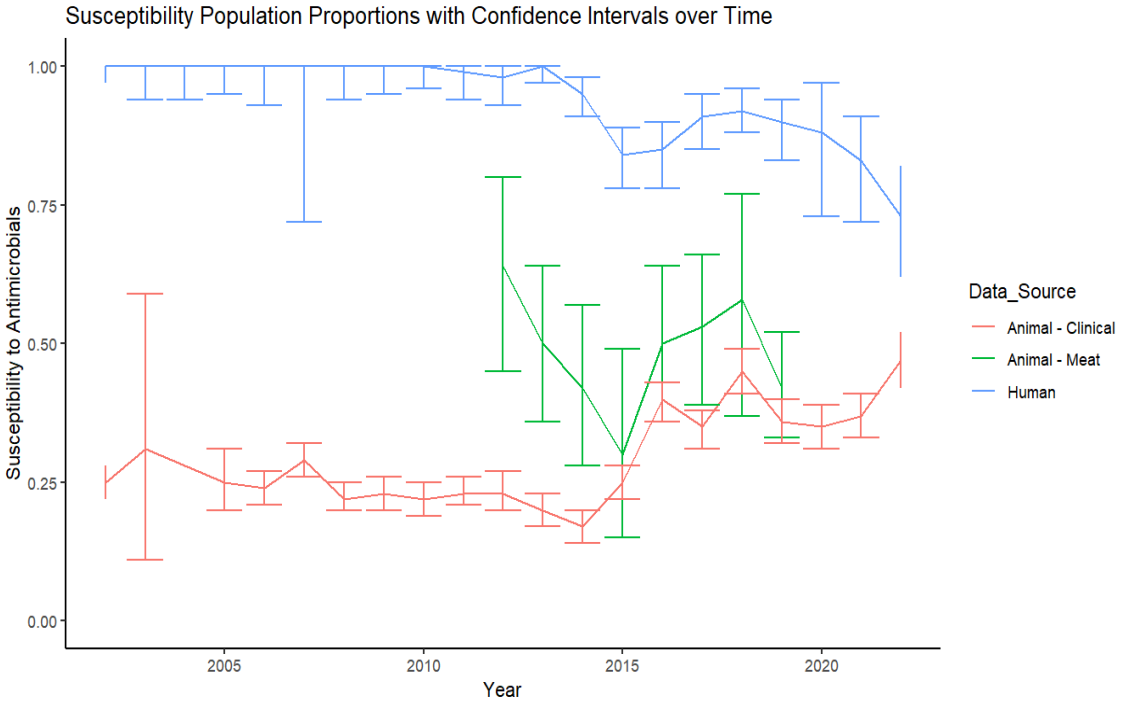

Figure 13. Campylobacter spp. in Animal Meat (S vs RI)

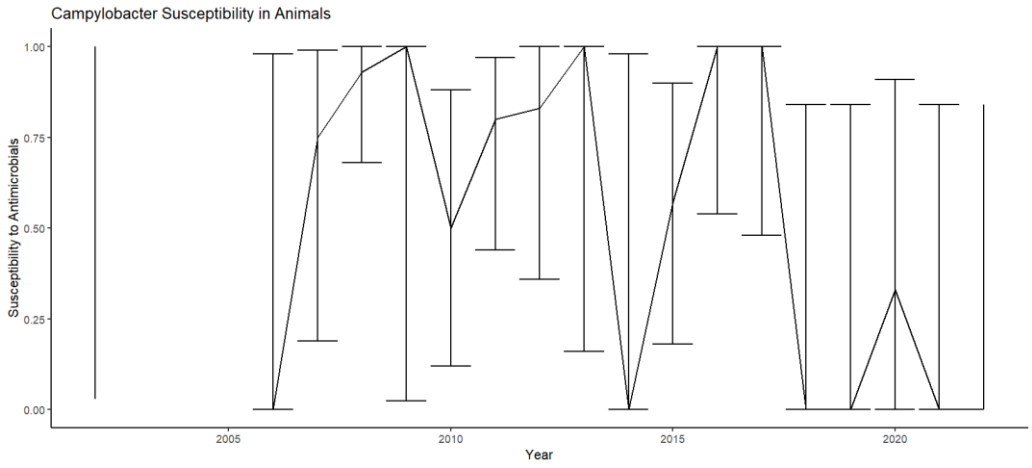

Figure 14. Salmonella spp. in Animals (S vs RI)

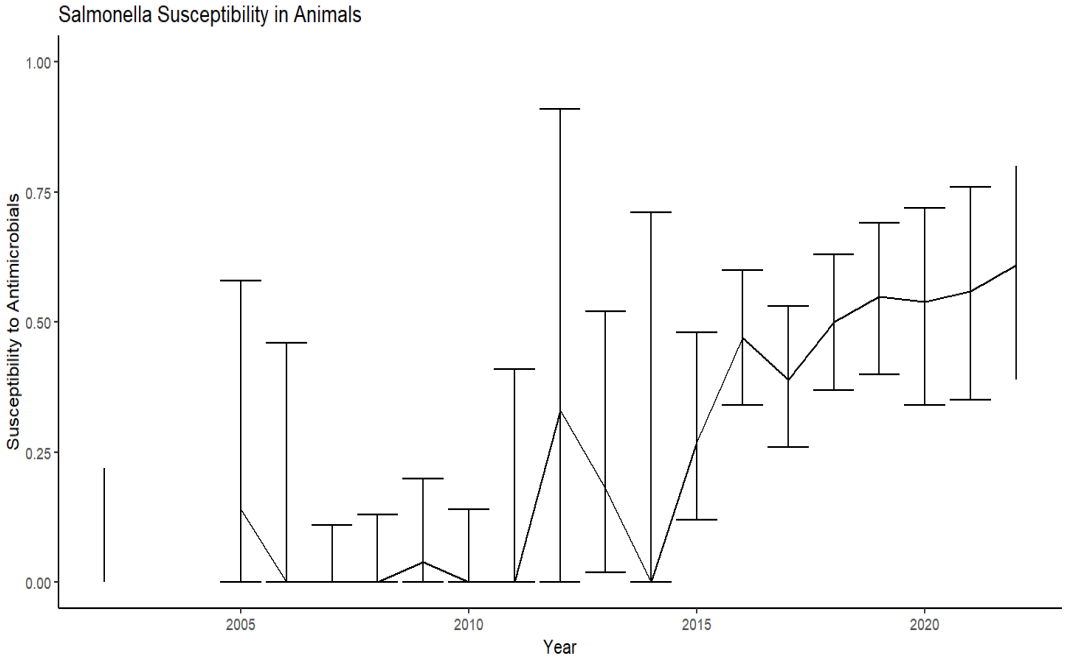

Figure 15. Susceptibility of Salmonella spp. by Source Type (S vs RI)