Characterizing Antimicrobial-Resistant Enterobacteriaceae Reservoirs From Farm to Plate in California

By

BAKYTZHAN BOLKENOV

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Biology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

_____

Xiang Yang, Chair

_____

Xunde Li

_____

Maurice Pitesky

Committee in Charge

2023

ACKNOWLEDGEMENTS

I would like to express my gratitude to the faculty and staff of the Animal Science department who supported and provided countless fellowships that greatly helped me and my family.

First, I would like to thank my supervisor Dr. Crystal Yang for her support and encouragement during my Ph.D. study. She was crazy enough to take me, a foreigner without any background knowledge in animal biology, as a Ph.D. student and believed in me. Crystal helped me to think out of the box and to become an independent thinker. Her insights and dedicatoin have been invaluable in shaping this research work. Also, I mastered both meat quality and safety in her lab, which will benefit my academic career.

I am profoundly grateful Dr. Xunde Li for his beneficial feedback and guidance that have been vital in improving my research and dissertation. Many thanks to Dr. Maurice Pitesky for serving on my dissertation committee and for his valuable edits and suggestions that helped to improve this work.

I want to thank all the lab members for being helpful and supportive: Fred, Brad, Toni, Sudipta, Yuyana, and Brady. Also, I want to thank the people who helped me in my research: Megan Gaa and Kurtis Lavelle for their help in lab work and helping to get lab supplies, and Katie Lee for her valuable advice and feedback. Without the help and support of these people, this research would be hard to accomplish.

Lastly, I want to thank my parents, siblings, and my spouse. They believed in me and encouraged and supported me all the time during my studies.

ABSTRACT

Antimicrobial resistance (AMR) in bacteria is a natural phenomenon that has been developing for thousands of years. However, intensive use of antibiotics in humans and animals starting in the middle of the last century has resulted in increased AMR, which has become a global threat to public health. Excessive use and misuse of antibiotics in food animals are blamed for the emergence of AMR. In recent years, multidrug resistance (MDR) in bacteria has become a serious issue by limiting the treatment options, making hospital stays longer and healthcare costs more expensive. In some cases, infectious diseases caused by AMR are impossible to treat. Antimicrobial-resistant bacteria (ARB) and their genetic determinants can be transferred from animals to the environment, workers, and animal products such as meat. Governments and various global organizations have taken the AMR issue seriously and have started to implement measures to limit the spread of AMR. In the U.S., the National Antimicrobial Resistance Monitoring System (NARMS) was founded in 1996 to track Antimicrobial-resistant bacteria (ARB)in humans, animals, and retail food and identify resistance trends, patterns, and mechanisms to make efficient interventions and drug development. However, the exposure of farm workers has been largely neglected by government agencies, industries, and researchers. Among the ARB, antimicrobial-resistant *Salmonella* poses a serious threat to public health. California is the most populated state in the U.S. However, antimicrobial resistance patterns of *Salmonella* in various retail meats have not been examined adequately. Therefore, two studies were conducted: 1) characterize AMR patterns in a farm environment and identify possible transmission routes of ARB from the farm environment to the farm workers; 2) characterize AMR patterns of antimicrobial-resistant *Salmonella* in retail meat from California. In the first study, environmental and worker samples were collected from the Hopkins Avian facility of the University of California, Davis.

Overall, 5 types of environmental (fecal samples, cage and eggs swab from layer house (LH) and fecal samples and door handle swabs from floor house (FH)) and 2 types of worker's samples (outwear and boots swabs). Samples were processed to isolate *Salmonella* and Generic *Escherichia coli* (*E. coli)* to assess their prevalence and test for antimicrobial resistance using the microbroth dilution method. Additionally, *E. coli* and aerobic bacteria were counted to evaluate the overall bacterial load in the facilities and on worker's personal protective equipment (PPE). *Salmonella* was not detected in any of the collected samples. Generic *E. coli* was present in all the samples except LH cage and egg swab samples. Counts of *E. coli* and aerobic bacteria were higher in fecal samples from both houses compared to other samples. Thirty-five isolates out of one hundred tested isolates were resistant to one drug, 9 isolates were resistant to two drugs and 6 isolates were multidrug-resistant (MDR) (resistant to at least three or more drugs). Higher resistance in *E. coli* isolates was observed to ampicillin (15 %) and nitrofurantoin (13 %), among other tested drugs. Antimicrobial-resistant *E. coli* isolates from LH fecal samples, FH fecal and door handle swab samples shared similar antimicrobial resistance patterns with worker's outwear and boots swab samples. The study results showed that door handles of FH pose a high risk of exposure as the prevalence of ARB was high in isolates of *E. coli* from the door handle swabs. Moreover, our results demonstrated that workers' PPE can serve as a protective measure against the transmission of ARB to workers. In the second study, a total of 849 meat samples (chicken, pork, ground turkey and beef) were collected from Northern and Southern California. One hundred thirty-two *Salmonella* isolates were recovered from the meat sample. Antimicrobial susceptibility test (AST) and whole genome sequencing were conducted to identify antimicrobial resistance patterns of *Salmonella* isolates. The recovery rate of *Salmonella* was high in chicken samples (24.01%) compared to ground turkey (5.42%) and pork samples (3.08%) ($P < 0.001$). Ground beef

samples were not contaminated with *Salmonell*a. Prevalence of *Salmonella* was higher in meat samples with reduced antibiotic claim (20.35%) compared to conventional (11.96%) (($P < 0.001$). Out of 132 isolates, 32 isolates (24.24%) were susceptible to all the tested drugs, while 17 isolates (12.88%) were resistant to one drug, 69 isolates (52.27%) to two drugs and 14 isolates (10.61%) to three or more drugs. *Salmonella* isolates were more resistant to tetracycline and streptomycin compared to other drugs. Whole Genome Sequencing (WGS) identified a total of 24 antimicrobial resistance genes, including the *gyrA* mutation as a notable resistance mechanism, along with the identification of 23 plasmid replicons in *Salmonella* isolates. Among the plasmid replicons, IncFIB (pN55391) was detected in 7 MDR *S*. Infantis isolates. IncFIB (pN55391) has been linked to the worldwide dissemination of pESI-like mega plasmid carriage in an emerging S. Infantis clone in previous studies. WGS results showed that the correlation between phenotypic and genotypic resistance was very high (96.85%). Overall, this study characterized AMR patterns and trends in *Salmonella* from retail meat in California, which might be helpful for public health protection, infection control, and clinical decision-making.

KEYWORDS: antimicrobial resistance, occupational exposure, retail meat, *Salmonella*, *Escherichia coli*.

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CHAPTER 1

LITERATURE REVIEW

1.1. Antimicrobial resistance is a global threat.

Microorganisms, including bacteria, have developed mechanisms to protect themselves from the effects of antibacterial substances for several billion years (Saliu et al., 2017). Antimicrobial resistance (AMR) is the capability of microorganisms to survive the effects of antimicrobial agents via various mechanisms, such as a mutation in an existing gene or obtaining resistant genes through horizontal gene transfer (Acar and Rostel, 2001). Four main mechanisms of AMR in bacteria are limiting the uptake of a drug, modifying a drug target, inactivating a drug and active drug efflux (Reygaert, 2018). Additionally, there are two main biological pathways that enable the evolution and dissemination of resistance: vertical gene transfer and horizontal gene transfer (Hedman et al., 2020).

Although the widespread usage of antibiotics began in the middle of the last century after the discovery of penicillin by Alexander Fleming, antimicrobial resistance has been recognized as a global threat and one of the leading public health problems of the 21st century only over the last two decades (Zhang et al., 2006; Prestinaci et al., 2015). The World Health Organization's (WHO) first report on worldwide surveillance of AMR, which was released in April 2014, showed the seriousness of the AMR problem (Prestinaci et al., 2015)

Many factors are attributed to the emergence and spread of AMR, including overuse and misuse of antimicrobial drugs, poor sanitation, poor practice of disease prevention and control, lack of knowledge, public awareness, legislation and lack of innovation and development of drug resources (Grundmann, 2014; World Health Organization, 2018). However, overuse or misuse of

antimicrobial drugs is considered the leading cause of the emergence and spread of AMR or accelerated development of AMR (Marshall and Levy, 2011). Especially, the usage of antimicrobial drugs in food animal production has been considered as one of the contributors to the AMR problem (Unjiya, 2017; Reygaert, 2018; World Health Organization, 2019). In the U.S., approximately 2.8 million clinical infections and 35,000 human deaths occur annually (Centers for Disease Control and Prevention [CDC], 2020). According to an estimate, by 2050, AMR could be the leading cause of mortality, with 10 million deaths, surpassing cancer in the mortality rate (O'Neill, 2014). Besides taking polls on people's lives, AMR causes an economic burden to the patients. It makes diseases and hospital stays longer, medical costs higher, and treatments ineffective and sometimes impossible to treat (Cosgrove, 2006). Making exact estimates of the burden of AMR is challenging. According to the recent review by Naylor et al. (2018), the economic burden from AMR per case is around $21832 and over $ 3 trillion in GDP loss. The economic burden from AMR in the U.S.,as reported by the Centers for Disease Control and Prevention (CDC), is around 4-5 billion dollars every year. It is expected that by 2050, a total global economic cost of US$100 trillion will be attributed to AMR (O'Neill, 2016).

Long-term exposure to antibiotics might weaken the immune system in humans, cause digestive problems, and have carcinogenic effects (Hiraku et al., 2004). Some infectious diseases easily treatable with penicillin in the past now require second and third-line antibiotics due to AMR. Other medical areas, such as chemotherapy for cancer treatment, organ transplantation, hip replacement surgery, intensive care for pre-term newborns, and many others, depend on the availability of efficient antibiotic drugs. Infections triggered by MDR bacteria are the main contributors to morbidity and mortality in people undergoing the abovementioned procedures

(Prestinaci et al., 2015). For example, studies have shown high AMR rates in infections in patients with cancer and liver transplantation (Kawecki et al., 2014; Nesher and Rolston, 2014).

1.2.Antibiotic usage in the livestock industry

Antimicrobial agents are a semi-synthetic or synthetic substance that kills or inhibits microorganisms (Page and Gautier, 2012). Since the 1940s, antibiotic agents have been used in the livestock industry for disease prevention and treatment and growth promotion. Antimicrobial agents in livestock have yielded healthier and more productive animals with lower disease incidence and reduced morbidity while lowering the cost of animal food production (Oliver et al., 2011). However, this honeymoon period was short-lived, and these benefits have yielded adverse outcomes: overuse and misuse of antimicrobials in food animal production resulted in an accelerated increase in antimicrobial resistance, and food animals have become reservoirs of antibiotic-resistant bacteria (Tang et al., 2017; Ma et al., 2021). Antibiotic-resistant bacteria and their genetic determinants can be transferred from food animals to humans via direct or indirect contact or food chains (Singer et al., 2003; Marshall and Levy, 2011).

Increased demand for protein led to the global spread of intensive farming (Tiseo et al., 2020), and antimicrobial usage has become an essential part of intensive farming (Van Boeckel et al., 2015). Global food animal production increased 4-5-fold since 1961(Ritchie and Roser, 2018). In turn, intensive farming expansion has led to increased consumption of antimicrobials worldwide (Van Boeckel et al., 2015). Two-thirds of the total medically important antimicrobials in the U.S. are associated with food animal production (Prestinaci et al., 2015; Patel et al., 2020). In 2019 alone 11,000 tons of antibiotics were used in animal production (Xu et al., 2022). It is projected that the consumption of antimicrobials will rise by 67% by 2030 worldwide, and the rise is likely caused by expected consumer demand for livestock products as the global

population and affluent people are increasing in developed countries (Van Boeckel et al., 2015). According to the Bayesian regression framework conducted by (Van Boeckel et al., 2015), antimicrobial consumption is expected to increase in pigs and chickens compared to cattle.

Antibiotics in food animals can be divided into three categories: therapeutic use, disease prevention, and growth promotion (Aidara-Kane et al., 2018). Usage of antibiotics for disease prevention and growth promotion in food animals are distinct practices, and they are differentiated based on the purpose of usage, stage of lifecycle, timing of antibiotic administration, and dosage levels. For example, in disease prevention, antibiotics are administered at therapeutic levels (higher doses), while in growth promotion, antibiotics are administered at subtherapeutic levels (lower doses). Another example antibiotics for disease prevention are administered for a short period of time before outbreaks, while antibiotics for growth promotion are administered continuously during the animal's growth phase or over a long period (Sneeringer Stacy et al., 2015). However, there might still be a blurred line between these two practices. There are still possibilities to use antibiotics as growth promoters despite the U.S. banned growth promoters in 2017. For example, dosing food animals continuously with antibiotics for disease prevention has been used in large-scale farming (Jones, 2018). Antibiotics are administered via feed, water, or intramuscular injection (Kirchhelle, 2018). Antimicrobials are administered to the entire flock or group in intensive farming via feed or water. The purpose of this practice is to prevent the spread of the disease. However, this practice sometimes results in overuse or misuse of antimicrobials, increasing ARB in animals. Additionally, the occurrence of infectious diseases and usage of antimicrobials depends on endogenous risk factors and farmers' decision-making, which can be influenced by cost-benefit analysis, farmer's expertise, and behavior (Lhermie et al., 2017).

WHO has recognized the necessity of coordinated global efforts to mitigate the AMR spread and recommended avoiding using antimicrobials in food animal production (Prestinaci et al., 2015; Lhermie et al., 2020). New European regulations on veterinary medicine and medicated feed are expected to substantially reduce antimicrobial usage in food animal production throughout Europe in the future (More, 2020). In the U.S., the FDA has been regulating antimicrobial drug prescriptions by implementing stricter policies on using antimicrobials over the years. For example, in 2017, the FDA banned the usage of growth promoters in the production of food animals, and in the same year, it also required veterinary prescriptions for "important antimicrobials" defined as Veterinary Feed Directive (VFD) drugs. Therefore, banning antimicrobials as growth promoters in Europe in 2003, followed by the U.S. in 2017, helped to reduce antimicrobial consumption (Lhermie et al., 2017). For example, in the Netherlands, consumption of antimicrobials decreased by 70 % between 2009 and 2019, and the resistance of some species of bacteria decreased compared to the previous years (De Greeff et al., 2020). FDA has been developing strategies to reduce the usage of antimicrobials in food-animal production (Food and Drug Administration [FDA], 2018). For example, in 2015, the FDA updated the new animal drug regulations to put into practice the veterinary feed directive (VFD), and according to this update, VFD drugs have been allowed only under the professional oversight of a licensed veterinarian. Recent studies have shown the effectiveness of reducing the usage of antimicrobials in food animal production in taming AMR. For example, the literature review conducted by Tang et al. (2017) has shown that restricting antimicrobial usage in food animal production might reduce animal ARB by up to 39 %. However, with an increase in global population and wealthy people in developing countries, demand for animal protein is expected to increase, further challenging the combat to reduce AMR.

1.3. Critically important antimicrobials for human and veterinary medicine.

Critically important antimicrobials play an essential role in treating life-threatening infectious diseases in humans and animals, and they are considered the last line of defense against some serious infectious illnesses (Collignon et al., 2016). *The List of Critically Important Antimicrobials of WHO for Human Medicine (WHO CIA List)* was developed in 2005 and has been updated since, the latest being updated in 2018 (World Health Organization, 2019). Since its development, *the CIA List* has been the benchmark for food animal producers worldwide by providing essential guidance (Tang et al., 2017). WHO classified antimicrobials into three groups: important, highly important, and critically important. (World Health Organization, 2019). Most antimicrobial classes on the WHO CIA list belong to the "Critically Important" category, and fewer belong to other groups. Apart from the WHO CIA list, the World Organization for Animal Health (WOAH) created a CIA list, which is a list of essential antimicrobials for veterinary medicine. Additionally, the WHO encourages countries to have their own CIA list, and the U.S. Food and Drug Administration (FDA) created a CIA list that is equivalent to the WHO CIA list (Scott et al., 2019). The CIAs have been further classified into high-priority and highest-priority CIAs based on the number of people with infections for which limited antimicrobials are available and the rate of usage among high-risk groups in human medicine (More, 2020). The highest priority CIAs are the quinolones (including fluoroquinolone), 3rd and higher-generation cephalosporins, macrolides and ketolides, glycopeptides, and polymyxins (World Health Organization, 2019). High-priority CIAs are aminoglycosides, penicillins, ansamycins, penems, glycylcyclines, lipopeptides, monobactams, oxazolidinones, and mycobacterial drugs. Highly important antimicrobials are tetracyclines, amphenicols, cephalosporins (first and second generations), lincosamides, pseudomonic acids,

riminofenazines, steroid antibacterials, streptogramins, sulfonamides, sulfones. Important antimicrobials are aminocyclitols, cyclic polypeptides, nitrofurantoin, nitroimidazoles, and pleuromutilins (Scott et al., 2019).

Most antimicrobial classes are common in both veterinary and human medicine; nevertheless, the importance of some antimicrobials might differ based on species and application (Szmolka and Nagy, 2013). According to the World Health Organization's categorization, critically important antibiotics for human medicine are fluoroquinolones, third- and fourth-generation cephalosporins, macrolides, glycopeptides, and polymyxins (World Health Organization, 2019). Penicillin, macrolides, and fluoroquinolones are mainly used to treat human infections, while tetracyclines, penicillin, and sulfonamides are frequently used to treat food-animal infections (Ma et al., 2021).

Not long after its invention, penicillin was used to treat bovine mastitis to sustain the sustain milk supplies during World War II. In 1948 sulfaquinoxaline was used in poultry for the prevention of coccidiosis. In the U.S., antibiotics in livestock were first approved in 1951, giving birth to antibiotic-reliant large-scale food animal production operations (Patel et al., 2020). In recent years, in the U.S., antibiotic consumption in chicken production is decreased dramatically.

For example, in 2018, ninety-two percent of broilers were produced without using medically important antimicrobials (Patel et al., 2020). Penicillin and tetracycline are mostly used in pigs worldwide (Græsbøll et al., 2019). In 2012, tetracycline accounted for 41 % of total sold antimicrobials in the U.S. (Food and Drug Administration [FDA], 2017). Extended-spectrum cephalosporins, macrolides, fluoroquinolones, and polymyxins (colistin) are classes of CIA used

in pigs and cattle worldwide (Lhermie et al., 2020). In the U.S., 43% of all medically essential antimicrobials were consumed in cattle in 2016 (Food and Drug Administration [FDA], 2017) In 2017, WHO released recommendations on the utilization of medically important antimicrobials in animal agriculture to keep the effectiveness of medically important antimicrobials(Scott et al., 2019). In 2018, the WHO issued guidelines on using HP-CIAs in food-producing animals; the guidelines recommended not to use HP-CIAs for human medicine in treating food animals with infectious disease diagnoses (Aidara-Kane et al., 2018). These measures have been taken to fight antimicrobial resistance and preserve the effectiveness of CIAs.

1.4. Occupational exposure to antimicrobial resistance in animal farms

Occupational exposure of animal farm workers to AMR has been largely neglected and unrecognized due to different reasons such as scarce knowledge about AMR burden, lack of adequate regulations, lack or insufficiency of surveillance and monitoring, and economic factors (Castillo Neyra et al., 2012; Aworh et al., 2021; Xin et al., 2022). The chance of transmission of ARB or antibiotic resistance genes (ARG) from food animals to farm workers is very high based on previous research, which reported that occupational exposure poses a risk to farm workers (Klous et al., 2016). Specifically, ARB can be transmitted from animals to farm works through various ways, including direct contact with animals or animal feces or products, inhalation of dust or aerosols containing ARB or ARG, contact with ARB or ARG contaminated surfaces, equipment, tools, water, or food (He et al., 2020; Khan et al., 2020; Founou et al., 2021; Sazykin et al., 2021; Yan et al., 2021; Bai et al., 2022; Xu et al., 2022). Therefore, animal farm workers are at an elevated risk of acquiring and being carriers of ARB and ARG. Being exposed to ARB might pose risks to farm workers when injured, and bacteria might enter the wound, which can

lead to complications or severe illnesses among workers. Moreover, farmworkers might then further disseminate ARB to the public; therefore, identifying more AMR reservoirs and occupational exposure routes to AMR is crucial in mitigating AMR among farm workers.

At present, while there are studies that have addressed occupational exposure to ARB in farmworkers in swine, poultry, and cattle farms (Voss et al., 2005; Price et al., 2007; Aworh et al., 2021; Xin et al., 2022), the actual burden of AMR among farm workers who work in different areas of animal agriculture is largely unknown The studies referenced in the previous sentence reported that farm environment and/or dust or aerosols might be reservoirs of ARB that might be transmitted to farm workers. But still, there is a lack of knowledge about potential reservoirs of AMR in farm environments such as farm facility doors or cages. Moreover, the personal protective equipment (PPE) of workers at animal farms is a potential reservoir of ARB that has been largely neglected. To date, there are only a few studies that characterized antimicrobial resistance patterns from farm workers' PPE, such as outwear and boots (Byrne et al., 2022; Gharbi et al., 2023).

Also, there is a lack of literature that examines patterns and dynamics of AMR in animal farm environments. Identifying the specific factors that facilitate AMR transmission among animal farm workers is a necessary step toward mitigating exposure. Additionally, finding new reservoirs of AMR in farm settings and characterizing AMR patterns is very important. The systematic literature review conducted by Tang et al. (2017) revealed that ARB can be transmitted from food animals to farm workers, but still, there is weaker evidence of transmission of ARB from food animals and farm workers. Sufficient research will help to reduce the risks related to occupational exposure to AMR in farm settings. However, farm workers in different types of animal farming, depending on the type of animals, might have

different risks of exposure to ARB or ARG. Therefore, more research on occupational exposure of farm workers to AMR is crucial.

1.5. Retail meat as a reservoir of antimicrobial resistant bacteria.

Meat and meat products are vital energy and protein sources widely consumed worldwide (Godfray et al., 2018; Sudatip et al., 2021). Moreover, with the increasing world population and affluence in developing countries, meat consumption is increasing rapidly (Godfray et al., 2018). Chicken consumption is growing at a very fast speed due to its low production cost, low fat, and lack of cultural and religious barriers (Food and Agriculture Organization [FAO], 2015).

Food animals are considered one of the primary sources of ARB (antimicrobial-resistant bacteria) (Founou et al., 2021). ARB in food animals can be transmitted to meat during slaughter from the surrounding environment containing ARB, animal skin, cross-contamination equipment or workers, inadequate evisceration, and poor sanitation (Okolo, 1986; Hazards et al., 2021: Centers for Disease Control and Prevention [CDC], 2022). Contaminated meat with ARB serves as a vehicle for the transmission of AMR along the food chain (Founou et al., 2016). Consequently, meat consumption is one of the vital transmission pathways of ARB to enter humans (Schroeder et al., 2004).

The prevalence of ARB in retail meat varies depending on the meat type, bacteria present, country of origin, type of antimicrobial used in food animal production, type of production (conventional or organic), season, geographic location, meat types and production practices, regulations, surveillance programs, meat sampling methods and bacteria isolation protocols (Molva & Atabay, 2016; Ballash et al., 2021; Lee et al., 2022). Additionally, antimicrobial resistance prevalence is strongly correlated with the development statutes of countries as most of the developing countries have not banned the usage of antimicrobial agents as growth promoters

(Prestinaci et al., 2015). Every meat type is harbored by different types of bacteria, and different types of bacteria develop resistance to antimicrobials differently (Reygaert, 2018). For example, *Salmonella* is common in chicken and pork compared to other types of meat (Vo et al., 2006), and beef is a reservoir for Shiga toxin-producing *Escherichia coli* (STEC) (Beutin et al., 1993).

Among the different kinds of bacteria family, members of the Enterobacteriaceae family, including *Salmonella* and *E. coli*, are commonly associated with foodborne outbreaks worldwide and develop resistance to medically important drugs, especially MDR strains, which challenge the treatments of infectious diseases. For example, in China, between 1994 and 2005, 16.73 % of all foodborne disease outbreaks were caused by *Salmonella* and accounted for 22.16 of illnesses (Wang et al., 2007).

1.6. *Escherichia coli* and antimicrobial resistance in *Escherichia coli*

Escherichia coli is a gram-negative bacteria member of the *Enterobacteriaceae* family, commonly inhabiting human and animal guts (von Baum and Marre, 2005). Most of the strains of *E. coli* are commensal and they are unharmful to their host. However, commensal *E. coli* can become opportunistic pathogens by acquiring virulence factors and genetic elements and can trigger infections in the urinary tract or the sites of surgical wounds (Erb et al., 2007; Jnani and Ray, 2022). *E. coli* has been most prevalent in ground beef compared to other meat types. It is likely that *E. coli* present in intestinal tracts of cattle can be transferred during the slaughter or other meat handling processes. Moreover, the grinding process further contributes to the highest prevalence of *E. coli* in beef.

Eshireshia coli is considered the most common Gram-negative bacteria, which can potentially cause a wide range of clinical infections among the resistant bacteria (Paitan, 2018).

Over the last two decades, multidrug resistance (MDR) in *E. coli* has been an alarming worldwide issue in human and veterinary medicine (Boucher et al., 2009; Poirel et al., 2018; Mills et al., 2022). Especially, MDR of E. *coli* to therapeutically important drugs such as cephalosporins, fluoroquinolones, carbapenem, and colistin has been a serious problem since MDR in *E. coli* limits the effectiveness of these clinically essential, frontline antimicrobials. Also, Extended-spectrum β-lactamases (ESBLs), enzymes produced by some strains of *E. coli*, are a serious threat and lead to higher rates of treatment failures (Mills et al., 2022). In order to survive the constant toxic pressure of different antimicrobials provided to host the animals, *E. coli* uses MDR as a tool (Szmolka and Nagy, 2013). *E. coli* mainly uses efflux pumps and mobile-resistant mechanisms to cope with arrays of different antimicrobials. Previous studies reported that the most common MDR pattern in *E. coli* has been resistance to aminoglycosides, β-lactams, sulfonamides, and tetracyclines. (Bywater et al., 2004; Szmolka and Nagy, 2013; Zhou et al., 2022). Major resistant genes found in *E. coli* from animals were ampicillin (blaTEM-1), tetracycline [tet(A) and tet(B)], streptomycin/spectinomycin (aadA1 and strA/B), sulfamethoxazole (sul1), and trimethoprim (dfrA1) (Szmolka and Nagy, 2013).

It is known that horizontal gene transfer is the main factor in gaining MDR in bacteria (Tzouvelekis et al., 2012). *E coli* has a robust ability to acquire resistance easily due to genetic flexibility and adaptability to the environment (Erb et al., 2007; Szmolka & Nagy, 2013). Mobile genetic elements (i.e. plasmids, transposons, and integrons) are thought to carry the majority of resistance genes (Na'was et al., 2013).

The insident of antimicrobial resistance in *E. coli* from humans and food animals has been increasing steadily since half of the last century (Erb et al., 2007; Tadesse et al., 2012). Resistant rates in *E. coli* might differ depending on factors such as usage of antibiotics, virulence

factors, food sources, and molecular epidemiology (Erb et al., 2007; Collignon et al., 2009; Xiao et al., 2022; McCowan et al., 2022).

Since *E. coli* is ubiquitous in food animals and considered a potential reservoir of resistant determinants, *E. coli* has been used for surveillance and monitoring as an indicator of AMR in many countries, including the U.S. (Von Baum and Marre, 2005; Alonso et al., 2017; Szmolka and Nagy, 2013; Fukuda et al., 2021; Centers for Disease Control and Prevention [CDC]). For example, in the U.S., the CDC established multiple surveillance programs (FoodNet, NARMS, National Shiga Toxin-producing *E. coli* (STEC) Surveillance) to track the resistance patterns and trends of infections of *E. coli* (Centers for Disease Control and Prevention [CDC], 2021).

1.7. *Salmonella* and antimicrobial resistance in *Salmonella*

Salmonella is a gram-negative bacteria, a pathogen, and a member of *the Enterobacteriaceae* family. There are two major species of *Salmonella*: *Salmonella enterica* and *Salmonella bongori*.The gut and intestinal tract of humans and farm animals are the main niche of *Salmonella entrica* (Andino and Hanning, 2015). There are nearly 2600 *Salmonella* serovars (Trachsel et al., 2022). However, only a handful of serovars are responsible for foodborne illnesses. They are *S.* Enteritidis, *S*. Typhimurium, and S. Newport. For example, in the U.S. the most common serotypes *S.* Enteritidis and *S.* Typhimurium, are responsible for estimated 1 million foodborne illnesses and nearly 20 000 deaths every year (World Health Organization, 2022).

Salmonella enterica is a zoonotic pathogen and the leading cause of foodborne diseases, human morbidity, and mortality worldwide (Knodler and Elfenbein, 2019). *Salmonella* infection is a main public health issue worldwide, causing 93.8 million cases of gastroenteritis globally each

year, with 155,000 deaths (Ngogo et al., 2020; Gong et al., 2022). *Salmonella* is one of the major causes of foodborne illness in the U.S. According to the calculations of the (CDC) 1.35 million people are infected, 26,500 are hospitalized, and 420 die each year in the United States due to non-typhoidal *Salmonella* (Centers for Disease Control and Prevention [CDC], 2023). It is estimated that around 1 million cases of foodborne salmonellosis are not reported each year (Cosby et al., 2015). Moreover, foodborne Salmonella infections are an economic burden. For example, estimated expenses due to Non-Typhoidal (NTP) *Salmonella* exceed 14 billion dollars yearly in the U.S. (Scharff, 2010).

Most of the *Salmonella* infections have been linked to the eating of contaminated food animal products (Foley and Lynne, 2008; Hur et al., 2012). Poultry is considered a major reservoir of *Salmonella* among the meat products. Compared to other meat types, poultry has been the cause of more outbreaks associated with *Salmonella*. For example, it was reported that 35% of *Salmonella* outbreaks were caused by poultry, and 71% of it is due to chicken (Cosby et al., 2015). On the other hand, *Salmonella* prevalence in chicken eggs is relatively low (1-2%) in the U.S. (Centers for Disease Control and Prevention [CDC], 2023).

The emergence of AMR and the emergence of MDR *Salmonella* strains has made *Salmonella* infections in humans and food animals more difficult to treat and increased the economic burden on the healthcare systems (Alcaine et al., 2007; Dadgostar, 2019). The emergence of antimicrobial resistance in *Salmonella* to traditional antimicrobial agents has limited the treatment options and caused the usage of critically important antimicrobial agents such as quinolones, fluoroquinolones (e.g., ciprofloxacin), and third-generation cephalosporins (Bradley et al., 2011; Frasson et al., 2016).

Salmonella uses different mechanisms to survive the effects of different antimicrobial agents, such as the production of enzymes, efflux pumps, and receptor modification. For example, in order to inactivate the β-lactam class of antimicrobials, *Salmonella* produce β-lactamases enzyme to cope with tetracycline and chloramphenicol Salmonella use efflux pump mechanism (Croft et al., 2007; Foley and Lynne, 2008). Another way for acquiring the resistance is horizontal gene transfer in *Salmonella.* Plasmids can carry antibiotic resistance genes that spread these genes within species or between species of bacteria. Over the past years, several plasmid replicons were linked to MDR in *Salmonella* and *Salmonella* outbreaks around the world. For example, between 2017 -2019 in Northern America, *Salmonella* outbreaks were linked to Col440II- and ColRNAI-like plasmid replicons (Miller et al., 2020)

Antimicrobial resistance patterns and prevalence of resistance of *Salmonella* in retail meat differ by country and geographical location. This difference might be due to different factors such as regulations, antimicrobial usage policies, livestock production practices, veterinary practices, consumer preferences and demands, antibiotic stewardship, and funding mechanisms to develop new classes of antibiotics (Van Boeckel et al., 2015; Jansen and Anderson, 2018). For example, a study examining antimicrobial resistance in *Salmonella* in retail meat in the U.S. between 2002-2017 found that Salmonella isolates from Northwestern parts of the country were more resistant to antimicrobials (Nyirabahizi et al., 2020). Even in one country, antimicrobial resistance patterns change over time due to regulations, consumer demand and regulations, and advancement in veterinary medicine, research, and surveillance. For example, in the U.S., the Food and Drug Administration (FDA) implemented regulations and changes due to antimicrobial resistance concerns between 1977 and 2017. These changes include banning certain antibiotics, phasing out some antibiotics, banning antibiotics for growth promotion, and approving new

antibiotics (Briceno, 2005). Additionally, collaborations between stakeholders, researchers, industries, and consumers might affect changing antimicrobial resistance patterns in retail meat (Grill, 2021).

Recent studies and reports show that in the U.S., antimicrobial resistance patterns in retail meat in *Salmonella* depend on many factors, such as location antimicrobial usage claim (Yin et al., 2021; Nyirabahizi et al., 2020). For example, Nyirabahizi et al. (2020) reported that antimicrobial-resistant *Salmonella* was more prevalent in the Northeastern part of the U.S. compared to other parts of the U.S.

1.8. Whole genome sequencing for detecting antimicrobial resistance in bacteria.

Antimicrobial susceptibility testing (AST) for detecting antimicrobial resistance in bacteria has served well for over 90 years since its invention (McDermott et al., 2016). AST has been a foundation for therapy in medicine therapy and monitoring AMR trends (Hendriksen et al., 2019). However, the antimicrobial susceptibility tests (ASTs) have the following limitations: long turnaround times ranging between 24 and 72 h, only selective antimicrobial agents can be tested, sometimes failure to detect resistance mechanisms, yield false-negative results, errors arising in inoculum preparation or culture conditions (Stoesser et al., 2013; Tamma et al., 2019). Also, in some cases, assessing phenotypic resistance might not be sufficient to determine the risks of AMR. More advanced methods are needed to evaluate the underlying genetic factors and discover previously unknown resistant mechanisms, genetic variants, and mutations to control AMR and make decisions in therapeutic drug prescriptions (Stoesser et al., 2013; Gordon et al., 2020).

In recent years, with the cost of sequencing going down dramatically and quality improvement of sequencing, whole genome sequencing (WGS) has become an essential and

invaluable tool in tracing antimicrobial resistance, identifying resistance mechanisms, studying the evolution of resistance in real-time under a variety of conditions, design clinical trials, developing novel antibiotics and diagnostic tests (Roemer and Boone, 2013; Grundmann, 2014; Anjum et al., 2018). Countless studies, antimicrobial resistance monitoring programs, and government agencies worldwide have proved the effectiveness of WGS in confirming, tracking and controlling antimicrobial-resistant bacteria (ARB) (Harris et al., 2013). Additionally, WGS has allowed investigation of the history and spread of antibiotic resistance (Grad et al., 2014)

Every disruptive technology also has flaws that need improvement and WGS is not an exception. Some studies identified the flaws of WGS in detecting and tracking AMR. They are the following: despite the price decrease, WGS is still relatively expensive, the data analysis process is very complex and time-consuming, undiscovered resistant mechanisms may not be found by existing databases and some mobile genetic elements cannot be captured (Ellington et al., 2017).

Application of WGS in routine health care has been slow due to its high cost (Schwarze et al., 2020). It is expected that the cost of sequencing will go down further, and sequencing technologies will improve over time (Furchgott, 2022). Therefore, there is a great chance that WGS will be gradually adopted as a universal tool for tracking and controlling AMR around the world.

1.19. Limitations of studies that characterized patterns of antimicrobial resistant *Salmonella* in California.

California is the most populated state in the U.S., with a population of nearly 40 million people, and is the fifth economy in the world. The population is diverse, and the Southern part of

the state is densely populated compared to the Northern part of the state. California is the nation's #1 agricultural state and livestock commodities account for 27% of it (USDA, 2023; USDA Economic Research Service, 2023).

Among the foodborne pathogens, *Salmonella* has been the leading cause of outbreaks and hospitalizations. *Salmonella* serotypes vary depending on geographic locations or places which is why 1475 of all serotypes have been given location names (Gossner et al., 2016). This also means that antimicrobial patterns of *Salmonella* are different depending on geographic locations. To the best of our knowledge, only two studies characterized the antimicrobial pattern of *Salmonella* in California (Zhao et al., 2006; Lee et al., 2022). However, the study conducted by (Zhao et al., 2006) was conducted 17 years ago, and only two sites in California had been sampled. Additionally, since that that time, many regulations have been issued by the FDA, banning antimicrobials as growth promoters and prohibiting the counter sale of some antimicrobials. The study conducted by (Lee et al., 2022) has limitations because of the small sample size that prevented comprehensively characterizing *Salmonella's* antimicrobial resistance patterns in California. Therefore, there is an urgent need for a thorough characterization of resistance patterns of *Salmonella* in California.

1.10. Mitigation strategies, research gaps and future directions

WHO has been coordinating a global action plan on antimicrobial resistance to optimize the usage of antimicrobials in human and veterinary medicine. Also, the World Organization for Animal Health (WOAH) has devised a strategy for antimicrobial resistance and controlled use of antimicrobials in order to support the global action plan (More, 2020). Additionally, governments around the world also contribute to this plan by implementing a One Health approach to control and monitor AMR. For example, In 2007, the U.S. One Health Office was

established in order to promote the One Health approach. The collaborative actions between CDC, the FDA, and the USDA have played a crucial role in addressing antimicrobial resistance. NARMS was established as part of the One Health approach to track resistance in *Salmonella, Campylobacter*, *Escherichia coli*, *Enterobacteria*, and other foodborne bacteria by sampling food-producing animals, retail meats and infectiously diseased humans (McDermott et al., 2016). Surveillance plays a crucial role in controlling the spread of AMR (Premanandh et al., 2016). Identifying resistance patterns and mechanisms helps create timely and efficient AMR control strategies. The problem of AMR spread can be addressed by implementing and promoting the One Health approach (Sudatip et al., 2021). WHO's first global report on AMR surveillance showed that monitoring the AMR is very helpful for orienting treatment choices, understanding the evolving patterns of AMR, and determining priority areas for interventions. The need for more surveillance in many countries and areas undermines the possibility of therapeutic interventions (Prestinaci et al., 2015).

However, additional measures are necessary to address the AMR problem more effectively. Since stakeholders, industries, and consumers are involved in the farm to fork process, all these sides should work hand in hand with each other to solve AMR problems. Therefore, public awareness and education is very important measure to help tackle the issue. Another measure is stewardship of farm workers. Therefore, training and education of farm workers is a very important measure that should continue to be implemented across the livestock industry. Developing new antimicrobials and using existing antimicrobials in full capacity are other strategies to combat antimicrobial resistance that prevent infections and improve diagnostics (Anjum et al., 2018). Lastly, strengthening industrial and academic research on AMR and occupational exposure to AMR is very crucial. The research is necessary for several reasons.

First, research on AMR is the bedrock for the formulation of new drugs and improvement of existing drugs. For example, understanding mechanisms of resistance and how they develop is crucial for designing a new drug. Second, robust research is fundamental in improving surveillance systems and diagnostics because advanced surveillance methods and accurate diagnostics help to provide appropriate treatments and infection control actions. Third, collaborative actions of industrial and academic researchers can bring the knowledge and resources together to fight AMR, as academic researchers might have expertise and novel ideas that can be implemented by industrial partners who have resources for drug development. Finally, research on AMR is essential in raising public awareness and can cause governments to improve their policies on fighting AMR (Rogers et al., 2017; Rogers et al., 2020).

In conclusion, controlling, reducing, and optimizing antimicrobial usage are the main methods to fight AMR. Controlling and reducing the spread of AMR and optimizing antimicrobial consumption require collective, coordinated actions at local, regional, and international levels. Also, strengthening research is crucial in order to develop new drugs or improve existing drugs, improve surveillance systems and diagnostics, raise public awareness, and affect government policies.

SUMMARY

The literature review examined the existing literature on ways of transmission of antimicrobial resistance bacteria in the food chain. The literature review revealed that occupational exposure of farm workers to antimicrobial resistance has been neglected due to a variety of factors and need for more research to improve the awareness of stakeholders, farm owners and farm workers. With the growing concern of dissemination of AMR globally, occupational exposure needs serious attention to assist in combat with AMR. Additionally, the

literature review critically assessed the existing body of literature on antimicrobial resistance patterns of *Salmonella* inretail meat as this type of pathogen becomes very dangerous if acquired multi-resistance to antimicrobials. Despite being the most populous state in the U.S. and the leading agricultural producer in agriculture, antimicrobial resistance patterns in retail meat in California have yet to be adequately characterized. With the advent of the development of WGS, an opportunity has arisen to confirm phenotypic resistance detected by AST genotypically using WGS. This literature review also discussed the pros and cons of using WGS for tracking and monitoring AMR. In the end, AMR mitigation strategies and surveillance's vital role were discussed.

CHAPTER 2. Occupational exposure to antibiotic-resistant bacteria in a small-scale poultry farming

Abstract

Antimicrobial resistance (AMR) is becoming an urgent global public health threat. Exessive and misuse of antimicrobials in human medicine and food animal production have become the primary contributing factors to the AMR problem. The intensive poultry production system uses large amounts of antimicrobials to treat or prevent bacterial infections that can potentially increase AMR in the production environment. During the past few decades, considerable evidence has accrued that poultry and livestock operations farmworkers are exposed to antimicrobial-resistant bacteria (ARB) shedding in poultry feces. However, the potential routes of transmission of ARB to farmworkers are still unclear. Therefore, the objective of the present study was to characterize ARB in the production environment and from the worker's personal protective equipment (PPE) to identify potential transmission routes and réservoirs of ARB. A total of 70 samples were collected at the Hopkins Avian facility of the University of California, Davis (UC Davis) between June and September 2022. The facility has two houses: a layer house (LH) where adult layers were kept and a floor house (FH) where young chickens were kept after hatching until their age reached 10 weeks. Three types of environmental samples were collected from the LH: fecal samples (n=10) from the floor, cage swabs (n=10), and fresh egg swabs (n=10). Two types of environmental samples were collected from the FH: floor fecal samples (n=10) and front door swabs (n=10) of the pens. Two types of swab samples from the workers were collected: outwear swabs (n=10) and boot swabs (n=10). Samples were processed to isolate generic *Escherichia coli* (*E. coli*) and *Salmonella* to assess the prevalence of the two bacterial species. The populations of total aerobic bacteria were also determined. Additionally, two *E. coli* isolates from each positive sample

were randomly selected for antimicrobial susceptibility test to 23 antibiotics using the microbroth dilution method. No *Salmonella* were detected from the collected samples. Also, *E. coli* were not recovered from LH fecal samples and egg swabs. Counts of *E. coli* were significantly higher in fecal samples collected from LH (7.31 log CFU/g) and FH (7.97 log CFU/g) than in boot swab samples (4.03 CFU/g)), FH door swab samples (3.04 log CFU/g) and outwear swab samples (2.67 CFU/g) ($P < 0.05$). Thirty-five percent of *E. coli* isolates conferred resistance to one drug, 9% to two drugs, and 6 % to three or more drugs. There was no significant difference in resistance to at least one drug in isolates of FH door swabs (60 %) and FH fecal samples (45 %), LH fecal samples (25 %), and outwear samples ($P < 0.05$). However, isolates of FH door swabs (60 %) had significantly higher resistance to at least one drug compared to isolates of boots swab samples (15 %). *E. coli* isolates conferred higher resistance to ampicillin (15 %) and nitrofurantoin (13 %) ($P < 0.05$). Even though the avian facility did not use antibiotics to treat sick birds, ARB was found in fecal samples of both LH and FH swab samples. Also, some *E. coli* isolates found in workers' outwear and boots swabs showed resistance to tested antimicrobial drugs such as ampicillin, nitrofurantoin, cefoxitin, and cefuroxime. Overall, this study showed that ARB was present in a poultry production environment (floor feces, front door handles). Moreover, our results suggested that workers might be exposed to ARB via door handles, and the personal protective equipment of workers (outwear and boots) can serve as the first line of defense against the exposure to ARB.

Keywords: Occupational safety, antimicrobial resistance, *Escherichia coli*, aerobic bacteria, poultry facility

Introduction

Antimicrobial resistance (AMR) occurs when microorganisms survive by developing resistant mechanisms after exposure to antibiotics or without exposure to antibiotics by evolution (Knöppel et al., 2017; Reygaert, 2018). Continuous use of antimicrobial drugs for therapeutic purposes in food animal production and human medicine selects for antimicrobial-resistant bacteria (ARB) (Love et al., 2011). As a result, infections caused by ARB are increasing, which are difficult and expensive to treat (Economou and Gousia, 2015). Moreover, some infections due to AMR cannot be treated with existing drugs, leading to increased morbidity and mortality (Livermore, 2009). Therefore, antimicrobial resistance (AMR) is a serious public health issue (Economou and Gousia, 2015; Prestinaci et al., 2015). Approximately 2.8 million clinical cases of individuals infected by ARB result in 35,000 deaths annually in the U.S. (Center for Disease and Prevention [CDC], 2019). In addition, the estimated economic burden of AMR is $21,832 per case, which results in total costs of $20 billion to the U.S. healthcare system (Naylor et al., 2018).

The poultry industry is a substantial portion of food animal production where antibiotics have historically been widely used to treat sick birds and prevent disease (Nhung et al., 2017; Hedman et al., 2020). However, intensive poultry production in confinement in large-scale operations with flocks can increase the spread of ARB among the animal population and their surroundings, potentially posing health risks to workers (Maron et al., 2013).

A significant portion of zoonotic diseases and infectious disease outbreaks that make humans sick are caused by pathogens (Coburn et al., 2007). Antimicrobial-resistant genes (ARG) can be transferred through mobile genetic elements between zoonotic pathogens and commensal bacteria (Wang et al., 2021). Commensal bacteria can become pathogenic under certain

conditions. Commensal *Escherichia coli* (*E. coli)* is common in chickens, and *Salmonella* is a major pathogen in chickens (Golden and Mishra, 2020). Commensal *E. Coli* has shown resistance to a wide range of essential antimicrobials, and multidrug resistance of *E. coli* results in treatment failure (Manyi-Loh et al., 2018). Antimicrobial-resistant *Salmonella* has been shown to cause elevated morbidity and mortality among infected patients, with 212 500 infectious diseases and 70 deaths each year in the U.S. (Martin et al., 2004; Centers for Disease Control and Prevention [CDC]., 2019). Multidrug resistance in *Salmonella* causes more severe and prolonged illnesses in humans and animals that are harder and sometimes impossible to treat (Parisi et al., 2018).

It has been reported by many studies that animal farm workers can be exposed to ARB via direct contact with animals, through a contaminated environment, or due to poor hygiene (Angulo et al., 2000; Akwar et al., 2007; Spellberg et al., 2016; Thanapongtharm et al., 2016; Ding et al., 2022). However, occupational exposure of farmworkers to ARB has been largely neglected compared to other aspects of ARB research due to a lack of awareness of the farmworker's lack of resources and regulations (de Jong et al., 2022; Castillo Neyra et al., 2012). A few studies have examined the occupational exposure of poultry farmworkers to ARB; most of these studies were conducted in Europe (Stobberingh et al., 1999; van den Bogaard et al., 2001; Paul et al., 2019) and in other countries (Al-Ghamdi et al., 1999; Aworh et al., 2019; Mandal et al., 2022). In the U.S., very few studies have assessed poultry farm workers' risk for colonization with antimicrobial-resistant *E. coli*. The results of one of the found studies showed that gentamicin-resistant *E. coli* from worker's stool samples were 32 times higher compared with stool samples from community references (Price Lance et al., 2007). Despite the substantial research in establishing pathways for the spread of ARB, the transmission routes of ARB from

the poultry production environment to workers are still unclear (Williams-Nguyen et al., 2016; Koch et al., 2017). Besides, with the increasing prevalence of small-scale poultry farms (farms typically managed by families or small groups of farmers with limited land, capital, and labor resources) (89 % of all the farms are small farms in the U.S.) in the U.S. in recent years, and studies are needed to identify ARB transmission routes in these farms (USDA, 2020). Some studies have shown that workers in small farms do not follow biosecurity rules such as wearing personal protective equipment, washing hands with soup, and avoiding stepping into boot baths (Alam et al., 2019; Amass et al., 2000). Moreover, no studies examined occupational exposure and AMR patterns at university-owned small-scale poultry facilities in the U.S. Here, students and interns who are employed and their safety is a priority. Therefore, the objective of this study was to conduct a pilot study in a university-owned small-scale poultry production facility to characterize ARB phenotypes and identify potential transmission routes of ARB from the working environment to employees in the facility.

Materials and Methods

Sample collection

The samples for this study were collected weekly for ten consecutive weeks from the Hopkins Avian facility of the University of California, Davis (UC Davis) between June 2022 and September 2022. The facility had two houses: layer house (LH), where adult layers hens were kept, and floor house (FH), where young chickens were kept after hatching until their age reached ten weeks. In the LH, the birds were kept in cages as a group hovered above the floor with manure under the cage's concrete slab. The FH was divided into pens (around 20 chickens in each pen) with pine shavings as litter. A total of 70 samples were collected from the environment and employees (a detailed sampling plan was described later), who were

responsible for the following animal care in both houses: feeding, checking water, collecting eggs, nail trimming, and cleaning the houses. All samples were collected at the end of the workday, with employees exposed to the working environment for 5-6 hours before sample collection. The same employee worked in both houses on each duty period. Therefore the samples were collected only for one employee in each sampling week.

Three types of environmental samples were collected from the LH: a mix of feces and litter from the floor, cage swabs, and fresh eggs. Two types of environmental samples were collected from the FH: a mix of feces and litter from the floor front door swabs of the pens. Two types of samples were collected from employees at the end of the workday: outwear and boots swab. In order to collect fecal samples from LH, two rows of cages were chosen randomly each time, and ten pellets mix of feces and litter were collected by hand; then pellets were placed into a non-filtered Whirl Pak bag (Nasco, Modesto, CA, U.S.) and mixed by hand to homogenize the pooled sample. For FH, five pens were chosen randomly, and five pellets of a mix of feces and litter from each pen were collected by hand and placed into a non-filtered Whirl Pak bag (Nasco, Modesto, CA, U.S.) at each sampling point. The bag's contents were mixed by hand to homogenize the pooled sample. Five pens were chosen randomly, and the handles of each front door were swabbed using EZ-Reach™ sponge samplers (World Bioproducts, Libertyville, IL, U.S.). After swabbing, the sponges were placed into their original bags sealed, and the bags were placed on ice in coolers. Workers' swab samples were collected by swabbing the entire surface of workers' outwear and boots using EZ-Reach™ sponge samplers (World Bioproducts, Libertyville, IL, U.S.). Additionally, at each sample collection time, a tray with 30 eggs was chosen, and the surface of eggs was swabbed using one EZ-Reach™ sponge samplers (World Bioproducts, Libertyville, IL, U.S.).

Bacteria isolation

After sample collection, all the collected samples in plastic bags were transported on ice to the laboratory for further processing, including bacteria isolation and enumeration within 2 hours. Ten grams of the fecal and litter mix samples were put in a sterile filter bag (Nasco, Modesto, CA, U.S.), with 90 ml of Tryptic Soy Broth (Difco, Detroit, MI, U.S.). Afterwards, the bags were homogenized by hand for 3 minutes and incubated at 35°C for 24 hours. For swab samples, 10 ml of phosphate-buffered saline (PBS) (National Diagnostics, Atlanta, GA, U.S.) was added to each sponge bag. Then, the bags were homogenized for 40 seconds by hand. Homogenized mixtures were serially diluted into buffered peptone water (BPW; 0.1%; Difco; Becton, Dickinson and Company, Sparks, MD, U.S.) tubes. For the enumeration of aerobic bacteria and *E. coli*, 1 mL of contents from the bags with feces and sponges were serially diluted into the tubes, and appropriate dilutions were then plated onto *E. coli* and APC (Aerobic Plate Count) petrifilms (3M Microbiology, St. Paul, MN, U.S.). *Escherichia coli* petrifilms were incubated at 35°C for 24 hours and APC petrifilms were incubated at 35°C for 48 hours. After the incubation period, *E. coli* and aerobic bacteria were counted from the *E. coli* and APC petrifilms, respectively.

Three colonies (blue colonies with gas bubbles around) from the *E. coli* petrifilms per sample type were randomly selected and were streaked one more time onto MacConkey Difco™ Sorbitol agar (Becton, Dickinson and Company, Sparks, MD, USA). Colonies were inoculated into TSB culture and incubated at 37°C for 20-24 hours. Then, 667 µL of the overnight culture added to 333 µL of 50% glycerol (sterile) in a 2 mL screw top tube and gently mixed by vortexing. The glycerol stock tubes were put into an 80°C freezer until further characterization for antimicrobial susceptibility test (AST). Presumptive positives for *E. coli* were confirmed by

PCR using forward primer 5'-CCG ATA CGC TGC CAA TCA GT-3 and the reverse primer: 5′-ACG CAG ACC GTA GGC -CAG G-3 (Thermo Fisher Scientific, Waltham,MA, U.S.). PCR was conducted using n 25 μL reaction volumes. The reaction volumes consisted of 12.5 μL Dream Taq Green Master Mix, 9.5 μL sterile water, 0.1 μL of forward primer and 0.1 μL of reverse primer, and 2 μL of template DNA. Gel electrophoresis was used to verify PCR amplification.

For isolation of *Salmonella,* 100 mL of the overnight enrichment (incubated bags with 10 g samples and 90 ml of Tryptic Soy Broth) from the filter bags and the sponge bags were added to 900 uL of RV (Rappaport-Vassiliadis) broth and put to incubation at 42°C for 24 hours. Finally, one loopful of liquid inoculum from enriched RV tubes was plated onto XLT- 4 (Remel, Lenexa, KS, U.S.) plates.

Antimicrobial susceptibility testing

A loop of frozen *E. coli* stock was recovered on blood agar plates (BAP, Remel Inc, Lenexa, Kansas, U.S.) and incubated overnight at 37°C for 18-24 hours. The inoculum was prepared by suspending a few well-isolated colonies from the blood agar plate in a tube with 5 mL of demineralized water. The liquid mixture was adjusted to an optical density (OD) reading between 0.08 and 0.1 at 625 nm of a spectrophotometer (BioMate 3; ThermoSpectronic, Rochester, NY). Ten microliters of aliquot were transferred from the adjusted demineralized water suspension to a sterile 11 mL tube of Cation-Adjusted Mueller-Hinton Broth (Difco; Becton, Dickinson and Company, Sparks, MD, U.S.). Homogenized bacterial suspension (50 μL) was then dispensed into each well of a MIC Minimum Inhibitory Concentration) plate (Sensititre® GN2F panels for Gram-negative bacteria; Thermo Fisher Scientific, Cleveland, U.S.). The panel plate included 23 antimicrobial agents from 11 antimicrobial classes:

aminoglycosides (amikacin, gentamicin, tobramycin), penicillins (ampicillin, piperacillin), β-lactam/β-lactamase inhibitor combinations (ampicillin/sulbactam 2:1 ratio, piperacillin/tazobactam constant 4, Ticarcillin / clavulanic acid constant 2), monobactams (aztreonam), cephems (cefepime, cefoxitin, ceftriaxone), cephalosporins (cefazolin, cefotetan, ceftazidime, cefuroxime, cefpodoxime), quinolones (ciprofloxacin), Penems (imipenem, meropenem), fluoroquinolones (gatifloxacin), nitrofuran (nitrofurantoin), and sulfonamides (trimethoprim/sulfamethoxazole). Clinical and Laboratory Standards Institute (CLSI) breakpoints were utilized to interpret the results of the MIC (Minimum Inhibitory Concentration) test. The phenotypic data were presented as either susceptible or resistant, with intermediate results combined with resistant as one group for analysis.

Statistical analysis

The experimental design for the present study was a cross-sectional sampling design. ANOVA (One-way analysis of variance) test was utilized to compare the population of total aerobic bacteria and *E. coli* among the sample types. Fisher's exact test was performed to differentiate the prevalece of antimicrobial-resistant *E. coli isolates* (MIC test results) between the sample types. The prevalence was calculated by dividing antimicrobial-resistant isolate numbers in each sample type by the overall sample numbers in that sample type. ALl the the analysisof data was carried out using R statistical software (The R (4.1.2) Foundation for Statistical Computing, Vienna, Austria). An alpha level of 0.05 was selected to test the statistical significance.

Results and Discussion

The results of the present study showed that farm workers can be exposed to ARB in their working environment, and wearing personal protective equipment such as boots and outwear is a crucial measure to protect workers from exposure to ARB. The outcomes of the present study will help lay the foundation for a large-scale study to mitigate the risks of occupational exposure to ARB.

Salmonella was not recovered from any collected samples in the present study. In previous studies, *the Salmonella* recovery rate was very diverse (0.5 - 61%) from collected poultry environmental samples (Rodriguez et al., 2006; Giombelli and Gloria, 2014; Velasquez et al., 2018; Bailey, et al., 2001; Rothrock et al, 2021; Gutierrez et al., 2020; De Rezende et al., 2001). Such variability of prevalence in *Salmonella* in these studies might be caused by factors such as geographic location, season, farm environment, feed and water quality, farm size and type, antibiotic usage, sample collection methods, and farm biosecurity practices. Therefore, comparing prevalence results from different studies should be cautiously assessed, considering all the factors that might affect the outcome. For example, a study found that fecal samples in an organic poultry farm have lower *Salmonella* prevalence than a conventional poultry farm, 5.6% and 38.8%, respectively (Walid et al., 2010). Free range environment in poultry farms, natural diet, and strict biosecurity measures might cause the lower prevalence of *Salmonella* in organic poultry farms. However, the present study's absence of *Salmonella* in farm environments might be caused by study design, as including some variables might play a crucial role and may result in different outcomes. Additionally good farm management, biosecurity measures and external factors such as proximity to other farms might be potential confounders of not recovering Salmonella in the present study.

Generic *E. coli* were prevalent in 50 out of 70 collected samples, being detected across all sample types except LH cage and egg swab samples. The population of generic *E. coli* and aerobic bacteria counts are shown in Table 2.1. Both *E. coli* and aerobic bacteria counts were lower in outwear and boots swabs compared to LH and FH fecal samples ($P < 0.05$). However, both *E. coli* and aerobic bacteria counts for the FH door swab were not significantly different compared to outwear and boots swab counts ($P < 0.05$). Generic *E. coli* counts were higher ($P < 0.05$) in the fecal samples collected from LH (7.31 log CFU/g) and FH (7.97 log CFU/g) compared to FH door swab samples (3.04 log CFU/g). Similarly, aerobic bacteria counts were higher ($P < 0.05$) in fecal samples from both LH (8.76 log CFU/g) and FH (8.31 log CFU/g) followed by FH door swab (6.31 log CFU/g) samples and LH cage swab (4.76 log CFU/g) while egg swab samples had the lowest counts (4.10 log CFU/g).

Previous studies on *E. coli* prevalence from poultry farm environmental samples and worker's samples varied (66 - 92.27%) (Mandal et al., 2022; Ilyas et al., 2021; Tang et al., 2022; Adenipekun et al., 2015). As mentioned before, the diverse prevalence of recovery *of E. coli* from farm environments depends on factors such as geographical location, scale of farms, antimicrobial usage and cleaning and sanitation practices on farms (Morris et al., 2023; Huber et al., 2021). Counts of *E. coli* in fecal samples in the present study were similar to the findings of previous studies (De Rezende et al., 2001; Diarrassouba et al., 2007). Prior studies reported contaminations of egg surfaces with *E. coli* (15- 42%) (Akond et al., 2009; Khan et al., 2016; Hossain et al., 2021). In the present study, LH was cleaned frequently (every week), which might be the reason for the lower bacterial load in LH and possibly caused not discovering *E. coli* in cage and egg swabs.

Isolates from the FH door swab were resistant to 14 drugs, FH fecal sample isolates were resistant to 8 drugs, LH fecal sample isolates were resistant to 5 drugs, outwear swab isolates were resistant

to 4 drugs, and of boots swab isolates were resistant to 2 drugs (Table 2.2). The E. *coli* isolates from all types of samples were susceptible to amikacin, piperacillin / tazobactam constant 4, ticarcillin / clavulanic acid constant 2, ceftazidime, gatifloxacin, aztreonam, ciprofloxacin, imipenem, and piperacillin. The highest phenotypic resistance was observed for ampicillin (15/100, 15 %), followed by nitrofurantoin (13/100, 13 %) and cefoxitin (7/100, 7 %). A full antibiogram pattern of the antimicrobial susceptibility testing is presented in Table 2.

The most resistance to the same drugs was noticed in FH door swabs and worker's outwear swab isolates. Isolates of *E. coli* from FH door swabs and worker's outwear swabs were resistant to the same drugs, such as ampicillin, nitrofurantoin, cefuroxime and trimethoprim/sulfamethoxazole (Table 2.2).

Isolates from all the environmental sample types (LH fecal samples, FH fecal and door swab samples) and one isolate of boot swab samples were resistant to cefoxitin. All the environmental and worker's sample types were resistant to ampicillin.

Generic *E. coli* isolates from the FH door swab (12/20, 60 %) had a higher prevalence of resistance to at least one drug compared to isolates from the boot swabs (3/20, 15 %) ($P < 0.05$). However, there was no difference in the occurence of resistance to at least one drug between the isolates from FH feces (9/20, 45 %), LH feces (5/20, 25 %) and outwear swabs (6/20, 30 %) ($P < 0.05$) (Table 2.3).

Thirty five percent (35/100) of all the tested generic *E. coli* isolates were resistant to at least one drug, nine percent (9/100) to two drugs, and six percent (6/100) of isolates were resistant to three or more antimicrobial drugs. Most prevalent non multidrug resistant (MDR) pattern was AMP-NIT (n=6). Four isolates from FH door swab samples were MDR, one isolate from LH fecal samples, and one outwear sample was MDR (Table 2.3).

The present study observed the highest resistance in *E. coli* isolates for ampicillin (15/100, 15%) and nitrofurantoin (13/100, 13%). However, these levels of resistance rates are not considered high resistance rate since, in the medical community the, resistance rates 20-30% above are considered as the highest level of resistance in bacteria that raise concern for public health. These antimicrobials are essential for veterinary and human medicine (Hasan et al., 2011). The resistance prevalence to ampicillin was lower compared to other related previous studies(Al-Ghamdi et al., 1999; Li et al., 2007; Saidi et al., 2012; Adelowo et al., 2014; Dou et al., 2016; Nhung et al., 2017). In most of these studies, the high resistance of ampicillin in *E. coli* was due to increased use of this prescription drug for treatment and ampicillin is a commonly prescribed antimicrobial to treat a wide range of infections worldwide (KaushiK et al., 2014). Besides, factors such as the horizontal transfer of resistance genes from other bacteria species to *E.coli* and the production of enzymes degrade or modify ampicillin might be possible reasons for the increased resistance to ampicillin in *E. coli* (Poirel et al., 2018; Li et al., 2019). Resistance rates of *E. coli* for nitrofurantoin in the present study were consistent with findings of previous occupational expose-related studies in poultry farming (Van den Bogaard et al., 2002; Hasan et al., 2011; Aworh et al., 2021). Nitrofurantoin is not widely used as ampicillin, only to treat urinary tract infections. Therefore, the low prevalence of antimicrobial resistance in *E. coli* in the present study and previous studies is an expected outcome.

Our results showed a high prevalence of AMR in *E. coli* isolates from FH door swabs and fecal samples, suggesting that the FH environment might be a potential ARB or ARG reservoir and routes of exposure. Moreover, isolates from LH fecal samples and FH samples (fecal and door swab samples) shared similar antimicrobial resistance patterns with worker's outwear and boots sample isolates. Previous studies on occupational exposure of poultry farm workers to AMR

concluded that ARB could be transmitted from the farm environments to workers (Price et al., 2007; Cho et al., 2012; Aworh et al., 2019; Aworh et al., 2021; Ilyas et al., 2021). These previous studies compared antimicrobial resistance patterns in E. *coli* from environmental samples to worker's urine or stool samples and found that resistance patterns were similar. Additionally, some studies found ARB or resistance genes in farm dust or hand-wash water on farms (Luiken et al., 2020; Luiken et al., 2022; Mandal et al., 2022). A study conducted in Tunisia reported that antimicrobial-resistant *Campylobacter* was found in 3% of chicken farm workers boot samples, but researchers did not recover the bacteria from worker's outwear (Gharbi et al., 2023). To our best knowledge, the present study is the first study characterizing antimicrobial resistance patterns of *E. coli* in a poultry facility environment and comparing these patterns with farm worker's outwear and footwear. The present study shows the importance of using

personal protective equipment in reducing the spread of ARB or ARG from farm environments to workers. Additionally, the high prevalence of antimicrobial-resistant *E. coli* in door handles could suggest a potential risk of AMR transmission to farm workers. Because workers might touch their face or mouth after touching the door handles, we noticed that workers did not wear gloves, which might expose them to ARB. Therefore, wearing gloves and frequently sanitizing door handles is an important measure to minimize the risk of transmission of ARB or ARG, as biosecurity measures have been proven to play a crucial role in minimize the transmission of pathogens in farms (Mallioris et al., 2023).

Different sample matrices yield different prevalence outcomes. For example, fecal samples might have higher bacteria prevalence compared to surface swab samples, affecting overall prevalence significantly in a study. Additionally, in the present study, birds and workers did not receive antibiotics before and during the sample collection period, and consequently, overall

resistance prevalence was low (35 %), and our results support the findings of Tang et al. (2017) where authors systematically reviewed 181 studies and found out that restriction of antimicrobials in food-producing animals are associated with reduced ARB in these animals. However, previous research has shown that ARB can still be present and transmitted in facilities with no antimicrobial drug use (Davies and Wales, 2019); and our study found that the poultry facility might be a reservoir of AMR in which workers may be exposed to ARB. The present study cannot imitate intensive poultry production, where antibiotics are usually used to prevent, control the disease, and treat birds. However, antibiotic usage in food animals in Western countries, including the U.S., has changed over the years. Implementing the Veterinary Feed Directive (VFD) in the U.S. restricted certain antibiotics, and medically necessary antibiotics are allowed only with veterinary oversight and prescription. (Food and Drug Administration [FDA], 2017). Additionally, there might be differences in population density and management practices between small-scale and intensive large-scale poultry production.

In the U.S., a surveillance program, NARMS, was established in 1996 to monitor antimicrobial resistance in hospitals, retail meats, and food animals (Centers for Disease Control and Prevention [CDC], 2023b). However, currently, there is a lack of systematic surveillance of AMR in farmworkers. Additionally, there is a lack of awareness or education among the public and policymakers about the risks of AMR affecting farmworkers in livestock production (Wemette et al., 2021). Moreover, a large body of previous and current AMR studies and regulatory efforts mainly focused on food safety from farm to retail and have considered pathogens as a food safety issue (Rhoades et al., 2009; Castillo Neyra et al., 2012). The abovementioned facts might explain the scarcity of research or data related to occupational exposure of animal farmworkers in the U.S. Therefore, more research is needed to identify possible ARB routes from the farm environment to

workers in order to raise awareness of farmworkers and producers to avoid the risk of occupational exposure to AMR.

Conclusion

Our findings revealed that AMR patterns observed in environment samples collected at both poultry houses closely resembled those found in workers' PPE swab samples, suggesting a potential occupational exposure of workers to ARB. Additionally, the higher prevalence of antimicrobial-resistant *E. coli* in the front door swab samples suggests that the facility may need to clean or sanitize the doors of the pen/chicken houses more frequently to minimize the spread of these bacteria to the environment and employees via direct contact with the doors. More extensive research is needed to identify more potential transmission routes of ARB from large-scale poultry production system environments to workers using emerging novel technologies such as metagenomic sequencing, machine learning and AI, nanotechnology, microfluidics, and biosensors.

TABLES AND FIGURES

Table 2.1. Counts of commensal *Escherichia coli* and aerobic bacteria in collected samples from the small-scale poultry farm.

Bacteria type	**Layer house samples**			**Floor house samples**		**Employee samples**		**SEM**	***P*-value**
	Fecal samples	Cage swab samples	Egg swap samples	Fecal samples	Front door swab samples	Outwear swab samples	Boots swab samples		
E. coli counts, log CFU/g	7.31^{a}	0^{c}	0^{c}	7.97^{a}	3.04^{b}	2.67^{b}	4.03^{b}	0.36	<0.001
Aerobic bacteria count, log CFU/g	8.76^{a}	4.76^{cd}	4.1^{d}	8.31^{a}	6.31^{b}	5.96^{bc}	6.13^{bc}	0.43	<0.001

$^{a, b, c, d}$ Least square means within a row with different superscripts differ ($P < 0.05$).

Table 2.2. Percentage of *E. coli* isolates resistant to 23 antimicrobials from phenotypic susceptibility testing by sample type.

CLSI class	Antimicrobial agent	Layer house fecal samples (n=20)	Floor house fecal samples (n=20)	Floor House Door swab samples (n=20)	Outwear swab samples (n=20)	Boot swab samples n=20)	Total n/N (%)
Aminoglycosides	AMI	0	0	0	0	0	0/100 (0%)
	GEN	0	1(5%)	1 (5%)	0	0	2/100 (2 %)
	TOB	0	1 (5 %)	2 (10%)	0	0	3/100 (3 %)
β-lactam/β-lactamase inhibitor combinations	A/S2	0	1(5%)	2 (10%)	0	0	3/100 (3%)
	P/T4	0	0	0	0	0	0/100 (0%)
	TIM2	0	0	0	0	0	0/100 (0%)
Cephalosporins	FAZ	2 (10%)	0	1(5%)	0	0	3/100 (3 %)
	TANS	1 (5%)	0	1 (5%)	0	0	2/100 (2 %)
	FUR	0	0	1 (5%)	1 (5%)	0	2/100 (2 %)
	POD	2 (10%)	1 (5%)	1 (5%)	0	0	4/100 (4 %)
	TAZ	0	0	0	0	0	0/100 (0%)
Cephems	FEP	0	0	1 (5%)	0	0	1/100 (1 %)
	FOX	1 (5%)	2 (10%)	3 (15 %)	0	1 (5%)	7/100 (6 %)
	AXO	0	0	1 (5%)	0	0	1/100 (1 %)
Fluoroquinolones	GAT	0	0	0	0	0	0/100 (0%)
Monobactams	AZT	0	0	0	0	0	0/100 (0%)
Nitrofuran	NIT	0	0	8 (40%)	5 (25%)		13/100 (13%)
Quinolones	CIP	0	0	0	0	0	0/100 (0%)
Penems	IMI	0	2 (10%)	0	0	0	2/100 (2 %)
	MERO	0	0	0	0	0	0/100 (0%)

Penicillins	AMP	1 (5%)	2 (10%)	6 (30%)	4 (20%)	2 (10%)	15/100 (15 %)
	PIP	0	0	1 (5%)	0	0	1/100 (1%)
Sulfonamides	SXT	0	1 (5%)	2 (10%)	1 (5%)	0	4/100 (4 %)

Abbreviations: AMI – amikacin, GEN – gentamicin, TOB – tobramycin, A/S2 - ampicillin/sulbactam 2:1 ratio, P/T4 - Piperacillin / tazobactam constant 4, TIM2 - Ticarcillin / clavulanic acid constant 2, FAZ - cefazolin, TANS – cefotetan, FUR – cefuroxime, POD – cefpodoxime, TAZ – ceftazidime, FEP – cefepime, FOX – cefoxitin, AXO – ceftriaxone, GAT – gatifloxacin, AZT – aztreonam, NIT – nitrofurantoin, CIP – ciprofloxacin, IMI – imipenem, AMP – ampicillin, PIP – piperacillin, SXT - trimethoprim/sulfamethoxazole, MERO - Meropenem.

Table 2.3. Distribution of antibiogram patterns of E. *coli* isolates

Sample type	No. (%) of isolates out of 100 isolates	Antimicrobial pattern (No. of isolates)
Floor house door swab samples	12 (12%) [a]	POD (n=1) SXT (n=1) AMP-NIT (n=3) GEN-NIT-TOB-SXT (n=1) AMP-FEP-NIT (n=1) AMP-AXO-FOX-PIP-NIT (n=1) AMP-A/S2-FAZ-TANS-FOX-FUR-NIT (n=1) FOX (n=1) TOB (n=1) A/S2-NIT (n=1)
Floor house fecal samples	9 (9%) [ab]	POD (n=1) FOX (n=1) SXT (n=1) GEN (n=1) A/S2 (n=1) AMP-TOB (n=1) AMP (n=1) IMI (n=2)
Layer house fecal samples	5(5%) [ab]	FAZ-FOX-POD (n=1) FOX (n=1) FAZ (n=1) AMP (n=1) TANS-POD (n=1)
Outwear swab samples	6 (6%) [ab]	AMP-NIT (n=3) NIT (n=1) SXT (n=1) AMP-FUR-NIT (n=1)
Boots swab samples	3 (3%) [b]	FOX (n=1) AMP (n=2)
Total	35/100 (35%)	-

[a, b] Least square means within a column with different superscripts differ ($P < 0.05$).
Abbreviations: AMI – amikacin, GEN – gentamicin, TOB – tobramycin, A/S2 - ampicillin/sulbactam 2:1 ratio, FAZ - cefazolin, TANS – cefotetan, FUR – cefuroxime, POD – cefpodoxime, TAZ – ceftazidime, FEP – cefepime, FOX – cefoxitin, AXO – ceftriaxone.

CHAPTER 3. Antimicrobial resistance patterns of non-typhoidal Salmonella from Retail Meat in California

Abstract. Antimicrobial resistance (AMR) is an expanding problem in the United States and worldwide. Antimicrobials in food animal production may unintentionally select antimicrobial-resistant bacteria (ARB), which can be transmitted to humans through consuming contaminated animal products. Here, we assessed the phenotypic and genotypic resistance of non-typhoidal *Salmonella* from retail meat collected in California in 2019 for the National Antimicrobial Resistance Monitoring System (NARMS) retail food surveillance. A total of 849 different types of fresh meat were collected from randomly selected grocery stores in Northern and Southern California from January to December 2019. *Salmonella* isolates were subjected to serotyping, antimicrobial susceptibility testing, and whole genome sequencing (WGS) to characterize AMR patterns. The overall prevalence of *Salmonella* was 15.31%, and the prevalence was significantly higher in chicken samples (24.01%) compared to ground turkey (5.42%) and pork samples (3.08%) ($P < 0.001$). No *Salmonella* isolates were recovered from ground beef samples. The prevalence of *Salmonella* in meat samples (20.35%) with reduced antibiotic usage production claim was significantly higher than that in conventional meat samples (11.96 %) ($P < 0.001$). *Salmonella* isolates were classified into 25 serotypes, with *S.* Kentucky (47.73%), *S.* Typhimurium (11.36%), and *S.* Alachua (7.58 %) as predominant serotypes. Thirty-two (24.24%) out of 132 *Salmonella* isolates were susceptible to all the tested antimicrobial drugs, while 75.76% were resistant to one or more drugs, 62.87% to two or more drugs, and 10.61 % to three or more drugs. *Salmonella* isolates were highly resistant to antimicrobial drugs, were tetracycline (82/132, 62.12%) and streptomycin (79/132, 59.85%). Isolates from samples with reduced antibiotic usage production claim (57/69, 82.61%) exhibited

not significantly higher resistance to at least one drug than those with conventional production claim (43/63, 68.25%).

However, resistance to two and at least two drugs was significantly higher in the reduced antibiotic usage claim isolates than in conventional production claim isolates ($P < 0.05$). Resistance to at least three drugs was at the same level in the isolates from the reduced antibiotic usage claim (7/69, 10.15 %) and conventional production claim (7/63, 11.11 %). A total of 23 resistant genes, a D87Y mutation of GyrA, and 23 plasmid replicons were identified from resistant *Salmonella* isolates. The plasmid replicon IncFIB (pN55391), which has been reported to promote the dissemination of multidrug-resistant (MDR) *Salmonella*, was found in 7 MDR *S.* Infantis isolates. WGS results correlated with phenotypic resistance with an overall sensitivity of 96.85%. Three phenotypically resistant Salmonella isolates did not contain gentamicin-resistant genes. Data from Northern and Southern California in this study helped us to understand the trends of AMR of *Salmonella* in retail meat in the highly populous and demographically diverse state of California.

Keywords: Retail meat, *Salmonella*, antimicrobial resistance, whole genome sequencing, NARMS.

INTRODUCTION

The development of antibiotics in the 20th century was a groundbreaking advancement in medicine and one of the most significant advances in modern science (Marston et al., 2016). However, after a few decades, this great achievement has been compromised by the emergence and spread of antimicrobial resistance (AMR) (Ventola, 2015). AMR is the ability of microorganisms to protect themselves from the effects of antimicrobial agents via different mechanisms such as enzymatic inactivation, alteration of target sites, efflux pumps, reduced intake, horizontal gene transfer and formation of biofilms (Tenover, 2006; Dadgostar, 2019; Morrison and Zembower, 2020). Nowadays, AMR is a major global threat to public health (Dadgostar, 2019; Waseem et al., 2019). For example, AMR causes 2.6 million infections and 44,000 deaths each year in the U.S., while costing around 20 billion USD in healthcare and 35 billion USD in lost productivity annually (Centers for Disease and Prevention, 2019) .

Overuse and misuse of antimicrobials in humand medine and food animal production have been considered as major contributors to the emergence of AMR around the globe. Moreover, the World Health Organization (WHO) acknowledged the usage of antibiotics in food animal production as one of the leading causes of the development and spread of AMR (World Health Organization, 2015). Many studies have shown the assosiation between the usage of antibiotics in food animal production and the emergence of Antimicrobial-resistant bacteria (ARB) (Barton, 2000; Spellberg et al., 2016). ARB with their genetic determinants can be transmitted from food animal production to humans via various pathways such as direct contact with animals, environmental and air routes, cross-contamination, water and the food chain, and global trade (Wegener; Godijk et al., 2022; Jin et al., 2022). Among these transmission pathways, food chain is considered as critical. Previous studies documented that among animal food products, meat is a major reservoir of ARB and AMR in enteric bacteria such as *Campylobacter, Eschericchia coli* (*E.*

coli) and *Salmonella* is a serious threat (Schroeder et al., 2004; Pires et al., 2019; Ali and Alsayeqh, 2022). Non-typhoidal *Salmonella* (hereafter referred to as *Salmonella*) is a common and widespread pathogen that causes foodborne infections and outbreaks in the U.S. and around the world (Mølbak et al., 2006; Hendriksen et al., 2011; Lai et al., 2020). AMR in *Salmonella,* especially multidrug resistance (MDR) has become a serious health concern. For example, *Salmonella* resistance to critically important drugs such as extended spectrum cephalosporins, fluoroquinolones, and carbapenems limits treatment options and heightens the risk of morbidity and mortality among the patients to an estimated 40% (Crump et al., 2015; World Health, 2017; CDC, 2019; Bharat et al., 2021).

California is a highly populous and demographically diverse state in the U.S. with almost 40 million people which constitutes a major consumer market for retail meat products. Hence, research on the prevalence, distribution and AMR patterns of major foodborne pathogens such as *Salmonella* is integral to ensuring food safety and public health. The perpetual nature of bacterial populations to evolve over time highlights the importance of continuous monitoring of the trends of pathogens circulating in the food supply chain. Previously, we have characterized AMR of *Salmonella* from retail meat collected in California in 2018 (Lee et al., 2022). The aim of the current study was to characterize the AMR profiles of *Salmonella* isolated from retail meats in California using samples collected by NARMS routine surveillance in 2019. The specific objectives of the study were to assess the prevalence and phenotypic and genotypic AMR in various *Salmonella* serovars, to identify the resistance patterns of *Salmonella*, and to assess the correlation between Salmonella *AMR* phenotypes and genotypes.

MATERIALS AND METHODS

Sampling, sample processing, and isolation of bacteria

Fresh retail meat samples were collected twice a month from January to December 2019 as a part of the NARMS Retail Meat Surveillance. A total of 849 samples were purchased from randomly selected grocery stores in Northern (Alameda County, Contra Costa County, and San Francisco) and Southern (Los Angeles, Ontario, and Irvine) California. The sample types included skin-on/bone-in chicken, ground beef, ground turkey, and pork chops (Table 3.2). Meat samples in different packages (plastic bags, plastic film packaging, modified atmospheric packaging (MAP), vacuum packing, and paper wrapping) were placed on ice immediately after purchasing, transported to the lab in refrigerated conditions, and processed within 72 hours after collection.

Samples were processed according to the NARMS Retail Meat Surveillance protocol (Food and Drug Administration [FDA], 2019). Briefly, 50 g of each sample was placed into 250 ml buffered peptone water (BPW) (Becton Dickinson, Franklin Lakes, NJ, United States) in whirl-pak bags and massaged by hand for 3 minutes. After massaging, the homogenates were incubated at 35°C for 18–24 h. Then, 0.1 ml overnight enrichment was transferred to 10 ml RVR10 (Rappaport-Vassiliadis) and incubated at 42°C for 20-24 hours. The RVR10 enrichments were streaked onto XLT-4 (Remel, Lenexa, KS, United States). Two colonies of presumptive *Salmonella* based on colony morphology were then streaked onto blood agar plates (BAP) (Remel Inc, Lenexa, Kansas, United States) and incubated at 35°C for 20-24 hours. Presumptive *Salmonella* isolates were banked in Brucella broth with 15% glycerol tubes and shipped to the FDA's Center for Veterinary Medicine (CVM) for antimicrobial susceptibility test, whole genome sequencing (WGS), and other analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test of *Salmonella* isolates was performed using broth microdilution method at FDA-CVM using standard protocols (Food and Drug Administration [FDA], 2016). The NARMS Gram negative panel (Thermo Fisher Scientific, Waltham, MA, United States) of 14 antimicrobial drugs and the Clinical and Laboratory Standards Institute's (CLSI) breakpoints used to interpret resistance are as follows: amoxicillin-clavulanic acid (≥32/16 μg/ml), ampicillin (≥32 μg/ml), azithromycin (≥32 μg/ml), ciprofloxacin (≥0.12 μg/ml), cefoxitin (≥32 μg/ml), ceftriaxone (≥4 μg/ml), chloramphenicol (≥32 μg/ml), gentamicin (≥16 μg/ml), meropenem (≥4 μg/ml), nalidixic acid (≥32 μg/ml), streptomycin (≥32 μg/ml), sulfisoxazole (≥512 μg/ml), tetracycline (≥16 μg/ml), and trimethoprim-sulfamethoxazole (≥4/76 μg/ml). As CLSI's M100-S27 expanded the Minimum Inhibitory Concentration (MIC) range for ciprofloxacin, decreased susceptibility (≥0.12 μg/ml) was also identified for ciprofloxacin (Food and Drug Administration [FDA], 2021). Multidrug resistance is a resistance to three or more antimicrobial drugs (Food and Drug Administration [FDA], 2020).

Whole-Genome Sequencing

Whole-genome sequencing of *Salmonella* isolates collected between January and June 2019 was conducted by FDA-CVM. For isolates collected after June 2019, sequencing of isolates from Northern and Southern California was conducted at Contra Costa Public Health Laboratory and the University of California Davis, respectively. Extraction of DNA and library preparation was conducted using standard FDA-CVM protocols as previously described (Tyson et al., 2015; Lee et al., 2022). Then, the DNA library was sequenced (paired-end, 150bp) utilizing the Illumina MiSeq platform (Illumina, Inc., San Diego, CA).

Identification of Resistance Genes and Plasmid Replicons

Resistance genes were identified by using the methods described by Tyson et al. (2015). Perl scripts were utilized to detect hits (≥85% amino acid identity and ≥50% sequence length) from a reference database of compiled genes from the ResFinder (Center for Genomic Epidemiology, DTU, 2022), ARG-ANNOT (IHU Méditerranée Infection) (2022), and CARD (McMaster University) public databases (2022). Additionally, PlasmidFinder (Center for Genomic Epidemiology, 2022) was used to identify plasmid replicons (≥95% identity and ≥60% coverage).

Statistical Analysis

R statistical software (The R (version 4.2.1) Statistical Computing, Vienna, Austria) was used to do all the analysis. The statistical significance is defined at an alpha level of 0.05. Descriptive statistics for the prevalence of *Salmonella* and distribution of antimicrobial susceptibility test results were conducted in R. Data analysis was conducted on a total of 132 *Salmonella* isolates from 130 different meat samples due to two *Salmonella* isolates of different serotypes recovered from 2 pork samples. The reduced antibiotic use category is composed of samples with label claims of no antibiotic ever and/or organic in packages. The association between *Salmonella* prevalence in retail meat samples and sampling region, season, meat type, package type, label claim, and store types based on their size (wholesale markets, supermarket chains, and grocery stores) were assessed using Fisher's exact test. Post-hoc analysis was performed using the *cldList* function of the R package companion (Mangiafico, 2021). The correlation (sensitivity) between phenotypical and genotypical AMR was calculated by dividing the number of phenotypical antimicrobial-resistant *Salmonella* isolates by the number of *Salmonella* isolates with corresponding resistant genes. A heatmap of hierarchical clustering was generated utilizing the heatmap3. package in R (Zhao et al., 2021).

RESULTS

Prevalence of *Salmonella* in retail meat

In total, 849 retail meat samples were collected, including 479 chickens, 65 ground beef, 240 ground turkey, and 65 pork chops. *Salmonella* was recovered from 130 out of the 849 (15.31%) samples. The *Salmonella* recovery rate was higher ($p < 0.001$) in Southern California samples (28.38%) compared to Northern California samples (5.22%) (Table 1). Seasonality was not associated with *Salmonella* prevalence in retail meat samples. The prevalence of *Salmonella* in chicken samples (24.01%) was higher ($p < 0.001$) than inground turkey (5.42%) and pork chops (3.08%). No *Salmonella* was detected from ground beef samples (Table 3.1). Amongst chicken samples, *Salmonella* prevalence was higher ($p < 0.001$) in whole chicken carcass (55.10%) compared to other chicken cuts (Table 3.2). With respect to package types, *Salmonella* prevalence was higher ($p < 0.001$) in plastic bags and other types of packages (vacuum, roll, paper) compared to MAP and plastic film overwrapping packages. There was a difference ($p < 0.001$) in the prevalence of *Salmonella* in retail meats between conventional production (11.96%) and reduced-antibiotic use claims (20.35%). There was no difference ($p < 0.05$) in the prevalence of *Salmonella* between the samples packaged in stores (14.17%) and pre-packaged samples (15.82%). Also, there was no significant distinction ($p > 0.05$) in *Salmonella* prevalence between the samples collected from wholesale markers, supermarket chains, and grocery stores.

Distribution of *Salmonella* Serotypes

Across the 132 recovered *Salmonella* isolates, 25 serovars were identified (Table 3). The top four serotypes that accounted for 73.49% of all *Salmonella* isolates were *S.* Kentucky (47.73%), *S.* Typhimurium (11.36%), *S.* Alachua (7.58%), and *S.* Infantis (6.82%). Additionally, all the top four serotypes were from chicken samples. Sixteen different serotypes were identified in the chicken samples, while eight serotypes were found in turkey samples and four serotypes

were from pork chop samples. All the serotypes were from a distinct meat type except *S.* Reading, *S.* Saintpaul and *S.* Uganda, which were identified in both chicken and ground turkey samples. Two *Salmonella* isolates of different serotypes were recovered from two pork samples, with *S.* Derby and *S.* Worthington recovered from one sample and *S.* Berta and *S.* Johannesburg from the other sample. *S.* Kentucky was the most frequent serotype in both reduced antibiotic use claims (49.28%) and conventional production samples (46.03%). Eleven serotypes were identified from Northern California samples while twenty-one serotypes were identified from Southern California samples, respectively. *S.* Kentucky was the most commonly prevalent serotype in both Northern and Southern California samples (Table 3.3).

Antimicrobial Susceptibility Profiles

All the *Salmonella* isolates were susceptible to azithromycin and meropenem. The greatest resistance was observed on tetracycline (82/132, 62.12%), followed by streptomycin (79/132, 59.85%) and sulfisoxazole (26/132, 19.70%). Resistance to aminoglycosides (gentamicin and streptomycin) was observed in isolates recovered from chicken and ground turkey samples. More than half of isolates from chicken (73/115, 63.48%) and nearly half of the isolates from ground turkey (6/13, 46.15%) were resistant to streptomycin. Beta-lactams (cefoxitin and ceftriaxone) and beta-lactam combination agents (amoxicillin-clavulanic acid) conferred resistance to tested antimicrobials in isolates from chicken. Nine out of the ten ciprofloxacin intermediate resistance isolates were from chicken samples and one ciprofloxacin intermediate resistance isolate was from pork (Table 3.4).

The occurrence of phenotypic AMR in *Salmonella* isolates by label claim (reduced antibiotics use or conventional) and region (Northern and Southern California) was presented in Table 3.5. Resistance to aminoglycoside drugs (gentamicin and streptomycin) was detected in

samples with both label claims. Resistance to tetracycline was higher ($p < 0.05$) in isolates of samples with reduced antibiotics use claim (52/69, 75.36%) compared to those from conventional samples (30/63, 47.62%). However, no difference ($p > 0.05$) in resistance to streptomycin, ceftriaxone, and quinolones (nalidixic acid and ciprofloxacin) was observed between the isolates from samples with conventional and reduced antibiotics use claims (Table 3.5). In addition, resistance to ampicillin was higher ($p < 0.05$) in *Salmonella* isolates from Northern California (5/27, 18.52%) compared to *Salmonella* isolates from Southern California (5/105, 4.76%).

Nearly a quarter of *Salmonella* isolates (32/132, 24.24%) were susceptible to all antimicrobials tested, while 12.88% (17/132) isolates were resistant to one antimicrobial drug, 52.27% (69/132) to two antimicrobial drugs, and 10.61% (14/132) to three or more antimicrobial drugs. Among the 14 MDR isolates, 12 isolates were from chicken samples while the remaining 2 isolates were from ground turkey and pork samples. Two *Salmonella* isolates from chicken were resistant to nine antimicrobials, and 1 isolate also from chicken was resistant to eight antimicrobials (Table 6). No difference ($p > 0.05$) was found between isolates with reduced antibiotic claims (57/69, 82.61%) and conventional (43/63, 68.25%) in resistance to at least one drug, neither between reduced antibiotic claim (7/69, 10.14%) and conventional (7/63, 11.11%) in resistance to at least three drugs. However, resistance to two drugs was higher ($p < 0.05$) in reduced antibiotic claim isolates (45/69, 65.22%) than in conventional production claim isolates (24/63, 38.10%). Also, resistance to at least two drugs was higher ($p < 0.05$) in reduced antibiotic claim isolates (52/69, 75.36%) compared to conventional production claim isolates (31/63, 49.21) (Table 6).

Antibiogram profiles of *Salmonella* serotypes are presented in Table 3.7. STR (n=11) was the most common pattern of single drug resistance and all by *S.* Kentucky isolates. STR-TET (n=55) and FIS-TET (n=14) were the most common patterns of two-drug resistance and non-MDR

among the *Salmonella* isolates. Ten different MDR antibiograms were identified with CIP-NAL-STR-FIS-TET (n=3) as the most common pattern. *S.* Infantis accounted for five of these MDR patterns, *S.* Kentucky accounted for two, and *S.* Braenderup, *S.* Saintpaul, and *S.* Worthington each accounted for one MDR pattern. Tetracycline, streptomycin, sulfisoxazole, ciprofloxacin, ceftriaxone, and nalidixic acid were frequently observed as antimicrobial drugs in these resistance patterns.

Distribution of Antimicrobial Resistance Genes and Plasmid Replicons

Among the 132 *Salmonella* isolates, 23 unique antimicrobial resistance genes and *gyrA* mutation conferring resistance to eight antimicrobial classes were identified (Table 3.8). The most prevalent resistance genes were *aac(6`)-Iaa* (130/132, 98.48%), *aph(3'')-Ib* (70/132, 53.03%) and *aph(6)-Id* (70/132, 53.03%). These genes encode for aminoglycoside enzymes, and they are present in all meat types (chicken, ground turkey and pork). Meanwhile, the remaining 7 aminoglycoside resistance genes (*ant (3'')-Ia, aac(3)-IId, aph(3')-Ia, aac(3)-IVa, aph(4)-Ia, aac(3)-VIa and aadA13*) were less frequent and were detected in isolates from poultry. Beta-lactam resistance genes (*blaCMY-2, blaTEM-1C, blaTEM-1B* and *blaCTX-M-65*) were found in low prevalence among *Salmonella* isolates. Genes *blaCMY-2* and *blaCTX-M-65* were detected from chicken isolates, while *blaTEM-1C* was detected in a chicken and 2 ground turkey isolates and *blaTEM-1B* was identified from a ground turkey and a pork isolate. The sulfonamide resistance gene, *sul1*, was present exclusively in chicken samples, while *sul2* was present in 14 chicken samples and a pork sample. The frequency of tetracycline-resistant genes, *tetA* and *tetB*, were quite high, with a prevalence of 23.48% (31/132) and 38.64% (51/132), respectively. Gene *tetA* was present in isolates from all types of meat, while gene *tetB* was present in chicken isolates only. A *gyrA* mutation (D87Y) corresponding to quinolone resistance was detected in 9

isolates from chicken. Additionally, another quinolone resistance gene, *qnrB19*, was present in an isolate from pork. Florfenicol gene, *floR*, was detected in 5 isolates solely from chicken samples. Also, fosfomycin resistance genes (*fosA7* and *fosA3*) were discovered mostly in *Salmonella* isolates from chicken samples. Resistance gene *fosA7* was more dominant (9.09%, 12/132) compared to *fosA3* (0.76%, 1/132).

A total of 23 plasmid replicons were identified amongst *Salmonella* isolates in this study (Figure 1). A higher number of plasmid replicons corresponding to Inc plasmids (n=17) was observed compared to Col plasmids (n=5). The most prevalent plasmid replicons were IncX1 (77/132, 58.33%), IncFIB(AP001918) (51/132, 38.64%), IncFII (47/132, 35.61%), IncFII(29) (47/132, 35.61%), IncI1-I(Alpha) (42/132, 31.82%) and ColpVC (40/132, 30.30%).

The hierarchical clustering of *Salmonella* isolates by serotypes, meat types, sampling regions, resistance genes, plasmid replicons, and AMR phenotypes is presented in Figure 2. The main cluster was mostly comprised of *S.* Kentucky from chicken from Southern California that was resistant to tetracycline and streptomycin and positive for *tetB*, *aac(6`)-Iaa*, *aph(3'')-Ib* and *aph(6)-Id* genes and IncX1, IncFIB (AP001918), IncFII, and IncFII(29) plasmid replicons. *S.* Kentucky isolates from chicken from Northern and Southern California formed another cluster that was resistant to streptomycin and positive of *aac(6`)-Iaa*, *aph(6)-Id* genes and *aph(3'')-Ib* and IncX1, InchI2, and InchI2A plasmid replicons. *S.* Typhimurium from chicken from Northern and Southern California formed a cluster that was resistant to tetracycline and sulfisoxazole and positive for *tetA*, *sul2*, and *aac(6`)-Iaa* genes and IncC and Col(pHhAD28) plasmid replicons. *S.* Infantis isolates from chicken from Southern and Northern California formed a distinguished cluster that was resistant to tetracycline, streptomycin, nalidixic acid,

ciprofloxacin, and sulfisoxazole and positive for *tetA, aac(3)-Iva, aph(4)-Ia, ant (3'')-Ia, sul1, aac(6`)-Iaa,* and *gyrA* mutation genes and IncFIB(pN55391) plasmid replicon.

Phenotype and genotype correlation

Overall, a very high correlation was observed between phenotypic resistance and the occurrence of corresponding resistance genes, with sensitivity of 96.85%. Only two aminoglycoside-resistant isolates showed discrepancies. One isolate resistant to gentamicin and the other one resistant to streptomycin did not have the corresponding resistance genes (Table 3.9). Phenotypic resistance and genotypic resistance correlation were not calculated for some drugs (azithromycin, meropenem, and ciprofloxacin), as we did not find any phenotypic resistant isolates to these drugs.

DISCUSSION

The occurrence of *Salmonella* in retail meat in California (15.31%) in this study was higher than both the national average (8.25 %) (Food and Drug Administration [FDA], 2020) and that from the previous year in California (4.30 %, 2018) (Lee et al., 2022). This overall higher prevalence was likely due to higher recovery rate by 1) changes in the NARMS protocol (processing 50 g in 2019 compared to 25 g in 2018 and 2) the high number of whole chicken carcasses samples collected in Southern California (79/240, 32.92%) than Northern California (19/239, 7.95%) in 2019 (Table 2). Previous research has also reported a higher prevalence of *Salmonella* in whole chicken carcasses compared to cut samples of chicken (Capita et al., 2003; Abd-Elghany et al., 2015). In addition to the higher number and higher prevalence of *Salmonella* in whole chicken carcasses from Southern California, other chicken parts such as breasts, wings, and legs from Southern California also had a higher prevalence of *Salmonella* compared to those from Northern California, which collectively contributed to the

significant higher *Salmonella* prevalence in chicken in Southern California (39.58%, 95/240) in contrast to that in Northern California (8.37%, 20/239).

Meat types in this study were not collected in identical proportions: chicken samples – 479 (56.41%), ground turkey samples – 240 (28.26%), ground beef samples – 65 (7.65%), and pork samples - 65 (7.65%). The disproportionate distribution of samples among the meat types might affect the *Salmonella* recovery rate from different types of meat. For instance, a larger variety of chicken samples (various cuts) were purchased as compared to turkey and beef, where only ground samples were collected. Our data indicated the highest recovery of *Salmonella* was in chicken samples (24.01%), followed by ground turkey (5.42%) and pork samples (3.08%), and zero recovery from ground beef samples. The cause of the higher prevalence of *Salmonella* in chicken was aforementioned. With respect to prevalence in other types of meat, ground turkey samples were lower compared to the national average (12.00%), pork and ground beef samples were close to the national averages of 4.00% in pork and ~1% in ground beef (Food and Drug Administration [FDA], 2020). The high prevalence of *Salmonella* in chicken compared to other types of retail meat has been well-documented in previous studies (Shafini et al., 2017; Nyirabahizi et al., 2020; Xu et al., 2020). In general, chicken is a significant source of *Salmonella*, as contamination can potentially occur throughout the entire production chain, from farm to transportation, during processing in slaughterhouses, and on retail shelves (CDC, 2023; Zeng et al., 2021).

The prevalence of *Salmonella* in chicken also varied by U.S. states (Zhao et al., 2001; Cui et al., 2005; Golden and Mishra, 2020; Nyirabahizi et al., 2020). For example, Zhao et al. (2001) reported that higher prevalence in chicken might be caused by sample type (whole chicken carcass), while Nyirabahizi et al. (2020) found that regional factors may impact the prevalence of *Salmonella*. The current study revealed a notably greater prevalence of *Salmonella* in samples

with reduced antibiotics claim (69/339, 20.35%) compared to those from conventional production (61/510, 11.96%). These findings differ from previous NARMS data, which reported a slightly higher prevalence of *Salmonella* in samples with conventional production compared to those with reduced antibiotics claims. (Yin et al., 2021; Lee et al., 2022). One possible explanation might be attributed, at least in part, to the survival and propagation of *Salmonella* on these farms, which may be favored by conditions associated with reduced antibiotic use. For example, reduced antimicrobial use might result in fewer interventions to control bacterial infections on farms, and lack of routine antimicrobial treatments might increase the prevalence of *Salmonella*. Moreover, lack of antimicrobial use can affect the competitive balance between beneficial and harmful bacteria in the gut of animals, leading to an increased *Salmonella* prevalence (Dhaka et al., 2023). It is also worth noting that larger numbers of pork, ground beef, and ground turkey samples from conventional production had low or zero recoveries of *Salmonella*, which contributed to the overall low prevalence of *Salmonell*a in meat samples from conventional raise in our study.

More than 2,600 *Salmonella* serotypes have been identified, with specific serotypes frequently associated with severe illnesses (Li et al., 2021). The present study classified the 132 *Salmonella* isolates into 25 serotypes. Among these serotypes, those frequently implicated in foodborne illness are *S*. Typhimurium and *S*. Enteritidis. In the present study, both *S*. Typhimurium (n=15) and *S*. Enteritidis (n=2) were found in chicken samples. *Salmonella* Infantis (n=9) accounted for 64.29% of MDR isolates and was the most prevalent MDR serotype in retail meat in California in 2019, which was different than the national NARMS data. In 2019, the most common MDR *Salmonella* serotype was I 4,[5],12:i: which comprised 26% of nationwide MDR isolates (Food and Drug Administration [FDA], 2020). However, the rise of MDR *S*. Infantis caused the national average of MDR *Salmonella* strains in retail chicken to increase from 20% in

2018 to 32% in 2019 (Food and Drug Administration [FDA], 2020). In our study, all the *S.* Infantis isolates were from chicken samples, which was consistent with the national trend. Finally, the prevalence of MDR *Salmonella* isolates in our study in California (10.61%) was at the same level as the national average (10%) from the NARMS 2019 surveillance data (Food and Drug Administration [FDA], 2020).

In the present study, a high prevalence of resistance to tetracycline and streptomycin in *Salmonella* isolates from poultry samples was observed. This is consistent with the results of the NARMS retail meat surveillance in California in 2018 (Lee et al., 2022) and the NARMS national AMR data of *Salmonella* from retail poultry in 2008-2017 (Yin et al., 2021). Tetracycline has been commonly used in poultry farming to prevent and cure different poultry illnesses, such as respiratory problems, gut inflammation, and joint infection (Mehdi et al., 2018). Streptomycin, as one of the earliest aminoglycosides developed for combating bacterial infections in the poultry industry, has been utilized against various pathogens including *E. coli*, *Salmonella*, *Mycoplasma*, and *Staphylococcus* (Washko and Zeissig, 1957). A notable observation in the current study is that the majority of *S.* Infantis isolates (9/10) were resistant to ciprofloxacin and nalidixic acid despite restrictions in fluoroquinolone use in food animal production in the U.S. We attempted to determine the relationships between the occurrence of *Salmonella* resistance and the claims of antibiotics use. However, no significant difference was found between isolates with reduced antibiotic claims and conventional in single and multidrug resistance despite the fact that resistance to two drugs was higher in isolates with claims of reduced antibiotics than in isolates with claims of conventional production.

Whole genome sequencing (WGS) has been an essential tool for the characterization and confirmation of AMR in bacteria, especially in the identification of resistance mechanisms where

AST has limitations (Köser et al., 2014). Our results showed that genotypic resistance was highly corellated with phenotypic resistance, with a sensitivity of 96.85%. Only one of the three *Salmonella* isolates that exhibited resistance to gentamicin by AST lacked the corresponding resistance gene by WGS analysis. This discordance might be due to the presence of undetected AMR genetic determinants or misclassification of the isolate from AST (Seribelli et al., 2020; Yin et al., 2022). On the other hand, the ability of WGS to detect only known AMR genetic determinants highlights the importance of continuous traditional AST for comprehensive AMR assessment (Köser et al., 2014). Consequently, it remains valuable to incorporate both WGS and AST to assess AMR patterns in pathogens, particularly given the potential of new resistance genes continuing to emerge.

Plasmid replicons are essential genetic elements that play a crucial role in the dissemination of antimicrobial resistant genes within and between bacterial species (Carattoli, 2009). Therefore, the identification and characterization of plasmids can provide insight into the transmission potential of AMR genes between or within bacteria species (Supa-amornkul et al., 2023). In the present study, we discovered various plasmid replicons among various *Salmonella* serovars. Many of these plasmid replicons have previously been associated with AMR genes. The most frequent plasmid replicon observed (IncX1) was detected in 77 *Salmonella* isolates. Seventy-six of these isolates were from chicken samples, and one isolate was from the ground turkey sample. Previous studies reported plasmid replicon IncX1 being associated with beta-lactam, quinolones, and tetracycline resistance genes (Johnson et al., 2012; Rozwandowicz et al., 2018). Additionally, in the present study, plasmid replicon IncFIB(pN55391) was found in 7 MDR, ESBL-producing *S.* Infantis isolates, and all these isolates came from poultry samples. This is worrisome because previously, plasmid replicon IncFIB(pN55391) has been linked to *S.* Infantis clone with

large megaplasmid, which has been disseminating quickly in the U.S. and worldwide during the last nine years (Kürekci et al., 2021; Tyson et al., 2021). In the present research, all the MDR *S.* Infantis genes harbored a *gyrA* mutation that confers resistance to fluoroquinolone, and four MDR *S.*Infantis had the extended-spectrum beta-lactamase gene *blaCTX-M-65*. The fast spread of this MDR *S.* Infantis clone is concerning as it might undermine the existing treatment options to treat infections.

CONCLUSION

Based on the present NARMS retail food surveillance in Southern and Northern California, the prevalence of *Salmonella* was higher in retail chicken than in ground turkey, pork, and ground beef and higher in meat with claims of reduced antibiotic use than conventional production in California in 2019. The occurrence of single and multidrug-resistant *Salmonella* was not significantly associated with claims of antibiotic use in live animals. Furthermore, *S.* Kentucky was the most common serotype, while *S.* Infantis was the most common MDR serotype. Tetracycline and streptomycin continue to be the antimicrobials that *Salmonella* is mostly resistant to. The detection of plasmid replicon IncFIB(pN55391) indicated the spreading of MDR *S.* Infantis in poultry. In addition, the results of AST and WGS were highly correlated, which underscores the effectiveness of WGS as an alternative method for testing AMR in bacteria. Overall, this study highlights the importance of continuous retail food surveillance to monitor the trends of AMR in retail meat to protect food safety and public health.

TABLES AND FIGURES

Table 3.1. Prevalence of *Salmonella* in retail meat samples by sampling region, season, meat type, packaging, label claim, and store type.

Variable	*Salmonella* positive n/N (%)	*P*-value
Region		
Southern California	105/370 (28.38 %) [a]	<0.001
Northern California	25/479 (5.22 %) [b]	
Season		
Winter	35/220 (15.91 %)	
Spring	34/210 (16.19 %)	0.916
Summer	29/209 (13.88 %)	
Fall	32/210 (15.24 %)	
Meat type		
Chicken	115/479 (24.01 %) [a]	
Ground turkey	13/240 (5.42 %) [b]	<0.001
Pork Chops	2/65 (3.08 %) [b]	
Ground Beef	0/65 (0.00 %) [b]	
Packaging		
Other (vacuum, roll, paper)	32/141 (22.70 %) [a]	
Plastic bag	38/127 (29.92 %) [a]	
MAP (modified atmospheric packaging)	55/492 (11.18 %) [b]	<0.001
Plastic film	5/89 (5.62 %) [b]	
Label claim		
Reduced Antibiotic claim [*]	69/339 (20.35 %) [a]	0.001
Conventional	61/510 (11.96 %) [b]	
Packaged in Store		
Yes	36/254 (14.17 %)	0.603
No	94/594 (15.82 %)	
Store type		
Wholesale markets	17/114 (14.91 %)	
Supermarket chains	77/480 (16.04 %)	0.845
Grocery stores	36/255 (14.12 %)	

[a and b] Least square means within a column with different superscripts for each category differ ($P < 0.05$)

[*] Samples from packages claiming that meats were from organic or antibiotic free raised animals.

Table 3.2. Prevalence of *Salmonella* in meat cuts by sampling region.

Meat type	Region n/N (%)		Total n/N (%)
	Northern California	Southern California	
Chicken	**20/239 (8.37 %)**	**95/240 (39.58 %)**	**115/479 (24.01 %)**
Whole chicken	2/19 (10.53 %)	52/79 (65.82 %)[a]	54/98 (55.10 %)[a]
Chicken breasts	8/42 (19.05 %)	28/82 (34.15 %)[b]	36/124 (29.03 %)[b]
Chicken wings	5/48 (10.42%)	7/23 (30.43 %)[b]	12/71 (16.90 %)[bc]
Chicken legs	2/76 (2.63 %)	8/44 (18.18 %)[bc]	10/120 (8.33 %)[c]
Chicken thighs	3/54 (5.56 %)	0/1 (0 %)[c]	3/55 (5.45 %)[c]
Mixed parts of chicken	0/0 (0.00 %)	0/11 (0.00 %)[c]	0/11 (0.00 %)[c]
Ground Beef	**0/60 (0.00 %)**	**0/5 (0.00 %)**	**0/65 (0.00 %)**
Ground turkey	**3/120 (2.5 %)**	**10/120 (8.33 %)**	**13/240 (5.42 %)**
Pork Chops	**2/60 (3.33 %)**	**0/5 (0.00 %)**	**2/65 (3.07 %)**
Overall	**25/479 (5.22 %)**	**105/370 (28.38 %)**	**130/849 (15.31 %)**

[abc] Least square means within a column with different superscripts for chicken cuts differ ($P< 0.05$).

Table 3.3. Distribution of *Salmonella* serotypes by retail meat types, label claim and sample collection site.

Serotype	Retail meat types (no. of isolates)			Antibiotic usage-related production claims		Sample collection site		Total N (%)
	Chicken (n = 115)	Ground turkey (n = 13)	Pork (n = 4)	Reduced Antibiotic claim (n=69)	Conventional (n=63)	Northern California (n=27)	Southern California (n=105)	
S. Alachua	10 (8.70%)	0	0	9 (13.04%)	1 (1.59 %)	1 (3.70 %)	9 (8.57 %)	10 (7.58 %)
S. Anatum	1 (0.87%)	0	0	0	1 (1.59 %)	0	1 (0.95 %)	1 (0.76 %)
S. Berta	0	0	1 (25.00%) [b]	0	1 (1.59 %)	1 (3.70 %)	0	1 (0.76 %)
S. Braenderup	4 (3.48%)	0	0	2 (2.90%)	2 (3.17%)	0	4 (3.81 %)	4 (3.03 %)
S. Derby	0	0	1 (25.00%) [a]	0	1 (1.59%)	1 (3.70 %)	0	1 (0.76 %)
S. Enteritidis	2 (1.74%)	0	0	1 (1.45 %)	1 (1.59%)	1 (3.70 %)	1 (0.95%)	2 (1.52 %)
S. Hadar	0	4 (30.77%)	0	1 (1.45 %)	3 (4.76%)	1 (3.70 %)	3 (2.86%)	4 (3.03 %)
S. Indiana	0	1 (7.69%)	0	1 (1.45 %)	0	0	1 (0.95%)	1 (0.76 %)
S. Infantis	9 (7.83%)	0	0	6 (8.70 %)	3 (4.76 %)	3 (11.11 %)	6 (5.71%)	9 (6.82 %)
S. Johannesburg	0	0	1 (25.00) [b]	0	1 (1.59%)	1 (3.70 %)	0	1 (0.76 %)
S. Kentucky	63 (54.78%)	0	0	34 (49.28 %)	29 (46.03)	11 (40.74 %)	52 (49.52%)	63 (47.73%)
S. Lille	1 (0.87%)	0	0	0	1 (1.59%)	0	1 (0.95 %)	1 (0.76 %)
S. Montevideo	1 (0.87%)	0	0	0	1 (1.59%)	0	1 (0.95%)	1 (0.76 %)
S. Muenchen	0	1 (7.69%)	0	1 (1.45 %)	0	0	1 (0.95%)	1 (0.76 %)
S. Ohio	1 (0.87%)	0	0	0	1 (1.59%)	0	1 (0.95%)	1 (0.76 %)
S. Orion	0	1 (7.69%)	0	0	1 (1.59%)	0	1(0.95%)	1 (0.76 %)
S. Reading	2 (1.74%)	3 (23.08%)	0	1 (1.45 %)	4 (6.35%)	2 (7.41%)	3 (2.86%)	5 (3.79 %)
S. Saintpaul	1 (0.87%)	1 (7.69)	0	1 (1.45 %)	1 (1.59%)	0	2 (1.90%)	2 (1.52 %)
S. Schwarzengrund	1 (0.87%)	0	0	0	1 (1.59%)	0	1 (0.95%)	1 (0.76 %)
S. Senftenberg	0	1 (7.69%)	0	1 (1.45 %)	0	0	1 (0.95%)	1 (0.76 %)
S. Typhimurium	15 (13.04%)	0	0	11 (15.94%)	4 (6.35%)	4 (14.81 %)	11 (10.48%)	15 (11.36 %)
S. Uganda	1 (0.87%)	1 (7.69%)	0	0	2 (3.17 %)	0	2 (1.90%)	2 (1.52 %)
S. Worthington	0	0	1 (25.00) [a]	0	1 (1.59%)	1 (3.70%)	0	1 (0.76 %)
S. I 4:i:-	1 (0.87%)	0	0	0	1 (1.59%)	0	1 (0.95%)	1 (0.76 %)
SIIIa 13,23:g,z51:-	2 (1.74%)	0	0	0	2 (3.17%)	0	2 (1.90%)	2/132 (1.52 %)

[a] Isolates are recovered from the same Pork sample.
[b] Isolates are recovered from the same Pork sample.

Table 3.4. Distribution of phenotypic antimicrobial resistance in *Salmonella* isolates by retail meat type.

CLSI class	Antimicrobial Agent	No. (%) of resistant isolates of each meat type			Overall resistance prevalence
		Chicken (n=115)	Ground Turkey (n=13)	Pork[b] (n=4)	
Aminoglycosides	GEN	2 (1.74%)	1 (7.69%)	0	3 (2.27%)
	STR	73 (63.48%)	6 (46.15 %)	0	79 (59.85%)
B-lactam combination agents	AMC	1 (0.87%)	0	0	1 (0.76%)
Cephems	FOX	1 (0.87%)	0	0	1 (0.76%)
	AXO	5 (4.35%)	0	0	5 (3.79%)
Folate pathway antagonists	FIS	25 (21.74%)	0	1 (25.00 %)	26 (19.70%)
	COT	2 (1.74%)	0	1 (25.00 %)	3 (2.27%)
Macrolides	AZI	0	0	0	0 (0%)
Penems	MER	0	0	0	0 (0%)
Penicillins	AMP	6 (5.22%)	3 (23.08%)	1 (25.00 %)	10 (7.58%)
Phenicols	CHL	5 (4.35%)	0	0	5 (3.79%)
Quinolones	NAL	9 (7.83%)	0	0	9 (6.82%)
	CIP[a]	9 (7.83 %)	0	1 (25.00 %)	10 (7.58 %)
Tetracyclines	TET	75 (65.22%)	6 (46.15%)	1 (25.00 %)	82 (62.12%)

[a]Ciprofloxacin (CIP) values are presented for intermediate susceptibility which is 0.12 µg/ml.

[b] Four isolates with different phenotypic profiles were included from two pork samples.

Abbreviations: GEN gentamicin, STR streptomycin, AMC amoxicillin-clavulanic acid, FOX cefoxitin, AXO ceftriaxone, FIS sulfisoxazole, COT trimethoprim-sulfamethoxazole, AZI azithromycin, MER meropenem, AMP ampicillin, CHL chloramphenicol, NAL nalidixic acid, CIP ciprofloxacin, TET tetracycline

Table 3.5. Distribution of phenotypic antimicrobial resistance in *Salmonella* isolates by label claim and sampling region.

CLSI class	Antimicrobial Agent	Antibiotic usage-related production claims (No. (%) of resistant Isolates)		Sampling region (No. (%) of resistant Isolates)		Overall resistance prevalence
		Reduced (n=69)	Conventional (n=63)	Northern California (n=27)	Southern California ((n=105)	
Aminoglycosides	GEN	1 (1.45 %)	2 (3.17 %)	0	3 (2.86 %)	3 (2.27%)
	STR	46 (66.67%)	33 (52.38%)	13 (48.15 %)	66 (62.86%)	79 (59.85%)
B-lactam combination agents	AMC	0	1 (1.59 %)	0	1 (0.95 %)	1 (0.76%)
Cephems	FOX	0	1 (1.59%)	0	1 (0.95 %)	1 (0.76%)
	AXO	1 (1.45 %)	4 (6.35 %)	2 (7.41 %)	3 (2.85 %)	5 (3.79%)
Folate pathway antagonists	FIS	16 (23.19)	10 (15.87 %)	7 (25.93 %)	19 (18.10 %)	26 (19.70%)
	COT	1 (1.45 %)	2 (3.17 %)	2 (7.41 %)	1 (0.95 %)	3 (2.27%)
Macrolides	AZI	0	0	0	0	0 (0%)
Penems	MER	0	0	0	0	0 (0%)
Penicillins	AMP	3 (4.35 %)	7 (11.11 %)	5 (18.52 %) [a]	5 (4.76 %) [b]	10 (7.58%)
Phenicols	CHL	3 (4.35 %)	2 (3.17 %)	3 (11.11 %)	2 (1.90 %)	5 (3.79%)
Quinolones	NAL	6 (8.70 %)	3 (4.35 %)	3 (11.11 %)	6 (5.71 %)	9 (6.82%)
	CIP[*]	6 (8.70 %)	4 (6.35 %)	4 (14.81 %)	6 (5.71 %)	10 (7.58 %)
Tetracyclines	TET	52 (75.36 %) [a]	30 (47.62%) [b]	14 (51.85 %)	68 (64.76 %)	82 (62.12%)

[*]Ciprofloxacin (CIP) values are presented for intermediate susceptibility which is 0.12 µg/ml.
Abbreviations: GEN gentamicin, STR streptomycin, AMC amoxicillin-clavulanic acid, FOX cefoxitin, AXO ceftriaxone, FIS sulfisoxazole, COT trimethoprim-sulfamethoxazole, AZI azithromycin, MER meropenem, AMP ampicillin, CHL chloramphenicol, NAL nalidixic acid, CIP ciprofloxacin, TET tetracycline

[a and b] Least square means within a row with different superscripts for each category differ ($P< 0.05$)

Table 3.6. Prevalence of multidrug resistance in *Salmonella* isolates by meat type and antibiotic usage-related production claims.

N. of drugs	Overall n =132 (%)	Meat type			Antibiotic usage-related production claims	
		Chicken n=115 (%)	Ground Turkey n=13 (%)	Pork n=4 (%)	Reduced n=69 (%)	Conventional n=63 (%)
0	32 (24.24%)	25 (21.74 %)	5 (38.46%)	2 (50.00 %)	12 (17.39%)	20 (31.74%)
1	17 (12.88%)	14 (12.17%)	2 (15.38%)	1 (25.00 %)	5 (7.25%)	12 (19.05%)
2	69 (52.27%)	64 (55.65%)	5 (38.46%)	0 (0.00%)	45 (65.22%) [a]	24 (38.10%) [b]
3	1 (0.76%)	1 (0.87%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.59%)
4	4 (3.03%)	2 (1.74%)	1 (7.69%)	1 (25.00%)	1 (1.45%)	3 (4.76%)
5	3 (2.27%)	3 (2.60%)	0 (0.00%)	0 (0.00%)	3 (4.35%)	0 (0.00%)
6	2 (1.52%)	2 (1.74%)	0 (0.00%)	0 (0.00%)	2 (2.90%)	0 (0.00%)
7	1 (0.76%)	1 (0.87%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.59%)
8	1 (0.76%)	1 (0.87%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.59%)
9	2 (1.52%)	2 (1.74%)	0 (0.00 %)	0 (0.00%)	1 (1.45%)	1 (1.59%)
>1	100 (75.76 %)	90 (78.26%)	8 (61.54%)	2 (50.00%)	57 (82.61%)	43 (68.25%)
>2	83 (62.88 %)	76 (66.09%)	6 (46.15 %)	1 (25.00%)	52 (75.36%) [a]	31 (49.21%) [b]
>3	14 (10.61%)	12 (10.43%)	1 (7.69%)	1 (25.00%)	7 (10.15%)	7 (11.11%)

[a and b] Least square means within a row with different superscripts for each category differ ($P< 0.05$

Table 3.7. Distribution of antibiogram patterns by *Salmonella* serotypes

Serotype Name	No. (%) of isolates out of 132 isolates	Antimicrobial pattern (No. of isolates)
S. Alachua	7 (5.30%)	STR-TET (n=7)
S. Braenderup	1 (0.76%)	AMC-AMP--FOX-AXO (n=1)
S. Hadar	4 (3.03%)	STR-TET(n=4)
S. Infantis	9 (6.82%)	AMP-AXO-CHL-CIP[a]-NAL-STR-FIS-TET (n=1) AMP-AXO-CHL-CIP[a]-NAL-STR-FIS-TET-COT (n=2) AMP-AXO-CIP[a]-NAL-STR-FIS-TET (n=1) CHL-CIP[a]-NAL-STR-FIS-TET (n=2) CIP[a]-NAL-STR-FIS-TET (n=3)
S. Johannesburg	1 (0.76%)	CIP[a] (n=1)
S. Kentucky	55 (41.67%)	GEN-STR-FIS (n=1) GEN-STR-FIS-TET (n=1) STR (n=11) STR-TET (42)
S. Reading	4 (3.03%)	AMP (n=3) STR-TET (n=1)
S. Saintpaul	1 (0.76%)	AMP-GEN-STR-TET (n=1)
S. Schwarzengrund	1 (0.76%)	STR (n=1)
S. Senftenberg	1 (0.76%)	STR-TET (n=1)
S. Typhimurium	14 (10.61%)	FIS-TET (n=14)
S. Worthington	1 (0.76%)	AMP-FIS-TET-COT (n=1)
SI 4:i:-	1 (0.76%)	TET (n=1)
Total	100/132 (75.76%)	-

[a]Ciprofloxacin in the table indicates intermediate susceptibility.
Abbreviations: GEN gentamicin, STR streptomycin, AMC amoxicillin-clavulanic acid, FOX cefoxitin, AXO ceftriaxone, FIS sulfisoxazole, COT trimethoprim-sulfamethoxazole, AZI azithromycin, MER meropenem, AMP ampicillin, CHL chloramphenicol, NAL nalidixic acid, CIP ciprofloxacin, TET tetracycline.

Table 3.8. Distribution of antimicrobial resistance genes in *Salmonella* isolates.

Resistance Class	Genes	Meat type			Total n/N (%)
		Chicken (n=115)	Ground Turkey (n=13)	Pork (n=4)	
Aminoglycoside	*aac(6`)-Iaa*	113	13	4	130/132 (98.48 %)
	aph(3'')-Ib	64	5	1	70/132 (53.03 %)
	aph(6)-Id	64	5	1	70/132 (53.03 %)
	ant (3'')-Ia	10	1	0	11/132 (8.33 %)
	aac(3)-Iva	6	0	0	6/132 (4.55 %)
	aac(3)-VIa	2	0	0	2/132 (1.52 %)
	aac(3)-IId	0	1	0	1/132(0.76 %)
	aph(4)-Ia	6	0	0	6/132 (4.55 %)
	aph(3')-Ia	5	1	0	6/132 (4.55 %)
	aadA13	1	0	0	1/132(0.76 %)
Beta-lactam	bla_{CMY-2}	1	0	0	1/132(0.76 %)
	bla_{TEM-1C}	1	2	0	3/132(2.27 %)
	bla_{TEM-1B}	0	1	1	2/132 (1.52 %)
	$bla_{CTX-M-65}$	4	0	0	4/132 (3.03 %)
Folate pathway inhibitor	*dfrA14*	2	0	1	3/132(2.27 %)
Sulfonamide	*sul1*	11	0	0	11/132 (8.33 %)
	sul2	14	0	1	15/132 (11.36 %)
Tetracycline	*tet(A)*	24	6	1	31/132 (23.48 %)
	tet(B)	51	0	0	51/132 (38.64 %)
Quinolone	*GyrA(87)* mutation	9	0	0	9/132 (6.82 %)
	qnrB19	0	0	1	1/132(0.76 %)
Florfenicol	*floR*	5	0	0	5/132 (3.79 %)
Fosfomycin	*fosA7*	11	0	1	12/132 (9.09 %)
	fosA3	1	0	0	1/132(0.76 %)

Table 3.9. Sensitivity of phenotypic and genotypic antimicrobial resistance in *Salmonella* isolates from retail meat.

CLSI class	Antimicrobial Agent	No. of resistant isolates	No. of isolates carrying resistant genes	Sensitivity[a](%)
Aminoglycosides	GEN	3	2	66.67
	STR	79	78	98.73
B-lactam combination agents	AMC	1	1	100
Cephems	FOX	1	1	100
	AXO	5	5	100
Folate pathway antagonists	FIS	26	26	100
	COT	1	1	100
Macrolides	AZI	0	0	N/A[b]
Penems	MER	0	0	N/A[b]
Penicillins	AMP	10	10	100
Phenicols	CHL	5	5	100
Quinolones	NAL	9	9	100
	CIP	0	0	N/A[b]
Tetracyclines	TET	82	82	100
Overall		222	220	96.85

[a] Sensitivity was calculated by dividing the number of isolates carrying resistant genes to the number of resistant genes.

[b] Sensitivity was not calculated because isolates were not resistant to these drugs.

Abbreviations: GEN gentamicin, STR streptomycin, AMC amoxicillin-clavulanic acid, FOX cefoxitin, AXO ceftriaxone, FIS sulfisoxazole, COT trimethoprim-sulfamethoxazole, AZI azithromycin, MER meropenem, AMP ampicillin, CHL chloramphenicol, NAL nalidixic acid, CIP ciprofloxacin, TET tetracycline.

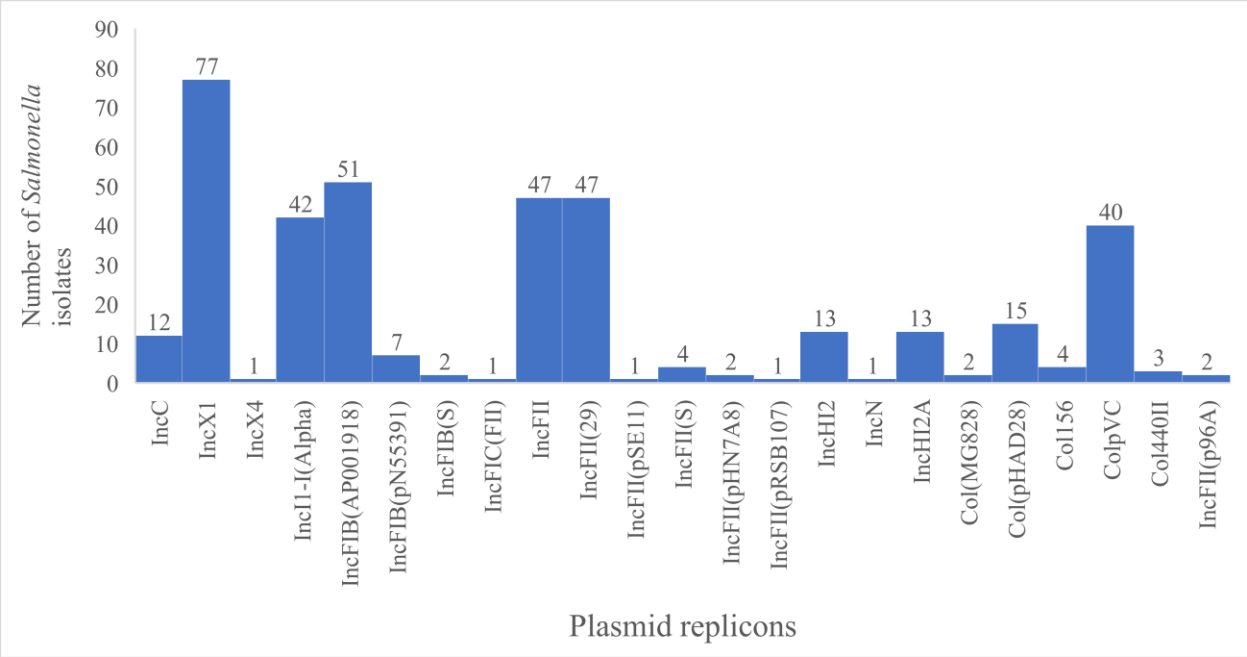

Figure 3.1. Distribution of plasmid replicons identified in *Salmonella* isolates (n=132).

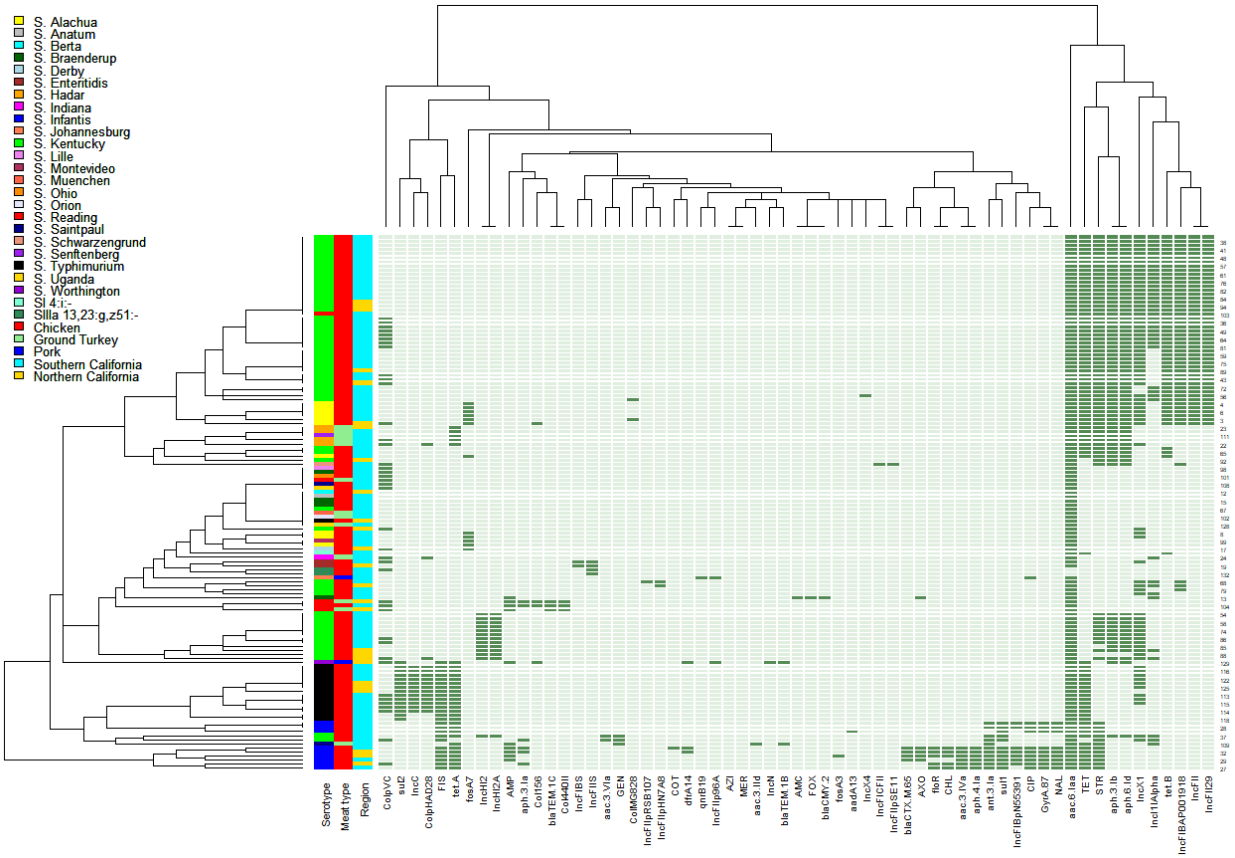

Figure 3.2. Heatmap and hierarchical clustering of *Salmonella* isolates by antimicrobial resistance genes (ARGs), plasmid replicons and phenotypic antimicrobial resistance (AMR). Dark green denotes presence of ARGs, plasmid replicons and AMR. Light green denotes absence presence of ARGs, plasmid replicons and AMR. Ciprofloxacin in the figure indicates intermediate susceptibility.GEN, gentamicin; STR, streptomycin; AMC, amoxicillin-clavulanic acid; FOX, cefoxitin; AXO,ceftriaxone; FIS, sulfisoxazole; COT, trimethoprim-sulfamethoxazole; AZI, azithromycin; MER, meropenem; AMP, ampicillin; CHL, chloramphenicol; NAL, nalidixicacid; CIP, ciprofloxacin; TET, tetracycline.

CHAPTER 4. GENERAL SUMMARY, DISCUSSION, AND CONCLUSION

Antimicrobial resistance has become a serious issue worldwide, challenging existing treatment options in human and veterinary medicine. Identifying the transmission routes of antimicrobial-resistant bacteria and the characterization of resistance patterns in antimicrobial-resistant bacteria is crucial in combatting the antimicrobial resistance problem.

The first study tried to identify probable transmission routes of bacteria from poultry farm environments to farm workers. According to previous studies, farm workers were exposed to antimicrobial resistance via direct contact with farm animals or indirectly through farm environments such as urine or feces, water, and soil. We isolated *E. coli* from environmental samples (feces, cage, and door handle surfaces) and worker's outwear and footwear samples. Then, antimicrobial patterns in *E. coli* isolates were characterized. The results showed that *E. coli* isolates from environmental and worker's samples shared similar resistant patterns, implying that antimicrobial-resistant bacteria might be transmitted to workers. The results also indicated that the door handles of the facilities pose a serious risk to worker's health, and worker's outwear and footwear an important defense to limit the transmission of ARB or ARG. Occupational exposure of farm workers to antimicrobial resistance has been long neglected, and further studies are needed to raise awareness among policymakers and farm workers.

The objective of the second study was to characterize antimicrobial resistance patterns in *Salmonella* from the collected retail meat samples in California. The study found that whole chicken samples had a higher prevalence of *Salmonella* compared to other chicken parts. Overall, *Salmonella* isolates from chicken samples were resistant to most of the tested antimicrobial drugs. Resistance to streptomycin and tetracycline was very high in *Salmonella* isolates. The multi-resistance pattern was most prevalent in *S.* Infantis isolates.

Phenotypical resistance in *Salmonella* was confirmed by using Whole Genome Sequencing (WGS), and WGS accurately found resistance in bacteria with a sensitivity of 96.85 %. Additionally, WGS allowed us to find plasmid replicons that play a crucial role in the transmission of antimicrobial resistance. The study identified resistance genes (*blaCTX−M−65, blaTEM-1C*, *GyrA(87)* mutation) and plasmid replicons (Col440II and IncFIB(pN55391)) which were associated with the previous outbreaks in North America. The study characterized antimicrobial resistance patterns in *Salmonella* and identified resistant genes and plasmid replicons that play crucial roles in the dissemination of AMR. Additionally, this comprehensive study helped to identify regional patterns of antimicrobial resistance in *Salmonella* and established a baseline understanding of the current resistance patterns. Moreover, the results of the study can be a foundation for future research, helping in tracking antimicrobial resistance changes over time. Finally, the results of the study can have implications for public health and policy decisions to limit the spread of AMR.

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