ABSTRACT

ABDELRAHIM, AFAF AHMED BASTAWY. Phenotypic and Genotypic Characterization of Antimicrobial-Resistant *Escherichia coli* Isolated from Multiple Retail Meat Types in North Carolina (2018-2019) (Under the direction of Dr. Siddhartha Thakur).

Surveillance of antimicrobial-resistant pathogens in U.S. retail meats occurs to determine their role as potential human transmission sources in the food chain. We conducted a phenotypic and genotypic analysis of antimicrobial-resistant *Escherichia coli* recovered from various retail meat types in North Carolina as an essential part of the National Antimicrobial Resistance Monitoring System (NARMS). From 2018 to 2019, 133 isolates of *E. coli* were recovered from a variety of meat products, including chicken (n=27), ground turkey (n=57), pork (n=24), and ground beef (n=25). We used the Sensitre™ broth microdilution system to determine phenotypic resistance to 14 antimicrobial agents and the Illumina next-generation sequencing platform for genotypic resistance profiling. The recovered isolates were divided into seven different phylogroups using the Clermont typing tool, with B1 (n=59, 44.4 %) and A (n=39, 29.3 %) being the most dominant, followed by B2 (n=14, 10.5 %), D (n=7, 5.3 %), F (n=6, 4.5 %), E (n=3, 2.3 %), and C (n=2, 1.5 %). Using MLST, 128 Sequence types (STs) were identified, including ST10, the most common ST (n = 16). The highest prevalence of *E. coli* isolates was found in ground turkey (n=57, 42.9%) and chicken (n=27, 20.3 %), followed by ground beef (n=25, 18.9%) and pork (n=24, 18%). Phenotypic and genotypic resistance was observed towards aminoglycosides, sulfonamides, beta-lactams, macrolides, tetracyclines, phenicols, and fluoroquinolones. Turkey meats were more resistant to all of the NARMS panel of tested agents than chicken, beef, or pork ($p < 0.05$). Importantly, all isolates from all sources were found to be susceptible to meropenem. A high percentage of turkey isolates (n=16, 28%) were multidrug-resistant (resistant to ≥ 3 antimicrobial classes) compared to 18.5% of chicken (n=5), 8.4% of pork (n=2), and 8% of beef isolates (n=2). This study highlights the benefit of antimicrobial resistance surveillance in identifying multidrug-resistant *E. coli* that can be epidemiologically tracked. This study highlights the benefit of antimicrobial resistance and surveillance to identify multidrug-resistant *E. coli* for epidemiologic tracking. Additionally, this study provided information on the extent of antimicrobial resistance; it is also a comprehensive report on the phenotypic and genotypic characterization of *E. coli* isolated from retail meats in North Carolina.

Keywords: Antimicrobial resistance, *E. coli*, Retail meats, Whole genome sequencing, Antimicrobial susceptibility testing

Phenotypic and Genotypic Characterization of Antimicrobial-Resistant

Escherichia coli Isolated from Multiple Retail Meat Types in North Carolina (2018-2019).

by

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DEDICATION

My dissertation is dedicated to my family, friends, and everyone who has guided and supported me on this journey.

BIOGRAPHY

Afaf Ahmed Bastawy Abdelrahim was born on the 14th of May, 1995 In Giza, Egypt which is also called the Giza Pyramid complex. In 2013 she attended the College of Pharmaceutical Sciences in Modern Sciences and Arts University (MSA). During her time as an undergraduate, she was an intern at multinational companies including Pfizer, GSK and AstraZeneca where she was awarded best achiever certificates in all of them. In her last year of undergraduate studies, she was exposed to research through a Microbiology project concerning the rapid detection of *Klebsiella pneumoniae*. At the end of the year, her project was chosen as the best one in the whole faculty by a professional panel from Greenwich university as well as Zewail university. In 2018, she graduated with a Bachelor of Science in Pharmaceutical Sciences and was hired as a teaching assistant at the Faculty of Dentistry at MSA university. Being interested in Microbiology and antibiotic resistance, in 2019, she was awarded the Fulbright scholarship to pursue a Master’s degree in Microbiology in the USA. In 2020, she joined the Plant and Microbial Biology department in the College of Sciences, North Carolina State University. Her research focused on the phenotypic and genotypic characterization of antimicrobial-resistant *E. coli* isolated from retail meats in North Carolina as part of the National Antimicrobial Resistance Monitoring System (NARMS) from 2018 till 2019 under the mentorship of Dr. Siddhartha Thakur.

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1.1 Introduction

Escherichia coli is a commensal microorganism that can be found in the intestine of both humans and animals. However, some *E. coli* strains are not safe because they can lead to serious infections (Ferens & Hovde, 2011). These strains can be classified into intestinal pathogenic *E.coli* and extraintestinal pathogenic *E. coli*. Many *E. coli* strains induce severe diarrhea leading to intestinal diseases. In addition, hemolytic *E. coli* can be caused by certain pathotypes including enterotoxigenic, enterohemorrhagic, and enteropathogenic *E. coli*. Serotype O157:H7 of EHEC *E. coli* is considered to be a zoonotic pathogen and it was previously reported that cattle can be a source of EHEC (O157:H7) (Kaper et al., 2004). Around 475,000 deaths annually are due to EHEC and 17 outbreaks that occur every year in North America take place because of EHEC (Fatima & Aziz, 2021). Extraintestinal *E. coli* (ExPEC) is a major pathogen that causes infections in humans and animals specifically urinary tract infections, but it can also lead to septicemia and meningitis (Poolman & Wacker, 2015). Uropathogenic *E. coli* can colonize the urinary tract causing pyelonephritis and cystitis that subsequently can lead to kidney failure. It was estimated that around 130-175 million humans get infected with UTI infections around the globe. Out of those infections, UPEC is responsible for 80% of the cases (Russo & Johnson, 2003). Additionally, the most common gram-negative bacterial pathogen that leads to meningitis in newborns is ExPEC (Bélanger et al., 2011).

From a biological point of view, resistance genes have similar qualities to virulence genes because both of them are necessary for a bacterium to be able to live in the environment (Beceiro et al., 2013). In order for pathogens to overcome antimicrobials, they acquired antimicrobial resistance in addition to having virulence factors to conquer human defenses mechanisms. All of that will help a bacterium in order to adjust and burgeon in its competitive surroundings. Bacterial annihilation in the environment is due to antibiotic pressure and human defenses of the immune system because they reduce bacterial growth and diversity (Feldman & Laland, 1996). Resistance genes and virulence factors can pass among species through horizontal gene transfer. Although mutations can be considered as a way for resistance dissemination, mobile genes' transfer is the principal mechanism (Burrus & Waldor, 2004).

Pathogenicity has a link to antimicrobial resistance such as the situations when an infection occurs by a biofilm-producing bacterium (Seral et al., 2003). Having virulence and resistance genes that can be either activated or repressed is common to take place through efflux pumps (Barbosa & Levy, 2000), porins (Tsai et al., 2011), cell wall changes (Moya et al., 2008), and two-component systems (Yeung et al., 2011). Opportunistic pathogens cannot induce infections in healthy individuals because they cannot invade the immune system of the host since they lack the necessary mechanisms for toxicity and invasion. Despite not being able to infect healthy individuals, opportunistic pathogens are able to induce infections in patients whose immune system is compromised. Such cases can be avoided and prevented through the utilization of antibiotic drugs. Antibiotic pressure can increase fitness and virulence in some species and in certain environments which will aid in their colonization (Beceiro et al., 2013).

1.2 Antimicrobial resistance

Prior to the discovery of antibiotics, a simple bacterial infection lead to certain death. All of that changed when antibiotics were discovered as they saved many lives and transfigured medicine. Unfortunately, these antibacterial agents have been accompanied by microorganisms that have developed resistance to them. Currently, medical professionals are worried about being on the threshold of the post-antibiotic era. When examining bacterial genomes, around 20,000 resistance genes have been identified nevertheless, the functional resistance genes are less in number (Wang et al., 2018).

The efficacy of antibiotics saved many people; however, this efficacy is currently in danger due to antibiotic resistance and resistant pathogens that are increasing all over the world. Overusing and misusing antibiotics is one of the causes for antibiotic resistance as well as the decreased efforts in research and novel drug production by the pharmaceutical companies (Lee Ventola, 2015). A certain number of pathogens have been identified by the CDC as a serious threat to public health and patients due to the antibiotic resistance phenomenon. In this regard, new regulations must be enacted as well as initiating research and providing management strategies to control this crisis (Barber & Sutherland, 2017).

1.3 Antibiotic-resistant *E. coli* in food animal production

Using antibiotics in food animal production has been a global concern and burden to public health. Antibiotics, regardless of their indication, are consistently used on farms. This leads to the selection and maintenance of resistant pathogens as reservoirs resulting in serious infections to humans. It may also lead to the transmission of mobile resistant genes among pathogens (Davis et al., 2018). Around 62% of the millions of pounds of antibiotics that are sold in the USA are utilized in food animal production (Yesserie, 2015).

E. coli is a common bacterium that is present in the intestine of vertebrates as well as a common contaminant of retail meats. In this regard, the US Food and Drug Administration tracks antibiotic-resistant foodborne *E. coli* as part of the National Antimicrobial Resistance Monitoring System (NARMS). From 1950 to 2002, it has been reported that the rates of resistance have been increasing in foodborne *E. coli* isolates. It has been also reported that isolates from livestock are growing quicker than the clinical isolates from humans (Tadesse et al., 2012). Even though there is a huge concern when it comes to antibiotic utilization in food animal production, the number of antibiotic agents sold in food animals increased annually from 2009 to 2015 (Yesserie, 2015).

When it comes to antibiotics utilization, food animal production practices and labeling of retail meats may be different. The laws indicated for antibiotic usage in animal production for slain animals are more severe specifically for meats labeled with “raised without antibiotics (RWA)” as well as “Organic” meats compared to conventional meat products. Nonetheless, Some antibiotic agents are allowed even under RWA and organic restrictions. For instance, organic labeled chicken begins on the "second day of life" and antibiotic usage is prohibited before reaching this stage. Additionally, RWA's criteria cover everything regarding the entire life cycle from "birth to harvest." (Connor, 2009). consequently, both RWA and organic standards allow for ovo antibiotic injections in combination with vaccinations (Davis et al., 2018).

There has been an increased demand for RWA and organic meat products as a result of consumers’ awareness regarding the relationship between antibiotic use in food animal production and the development of antibiotic resistance (Davis et al., 2018). It has been reported that bacteria retrieved from poultry that were conventionally raised show higher rates of antibiotic resistance as well as multidrug resistance than the isolates recovered from products raised without antibiotics usage or organically (Lestari et al., 2009), despite the fact that some exceptions have been reported (Millman et al., 2013).

1.4 Antibiotics used in livestock in relation to resistant *E. coli*

Since antibiotics are commonly used in livestock, they can be a reservoir for antibiotic-resistant pathogens as well as resistant genes (Roth et al., 2019a). In 2019, tetracyclines were the most sold antibiotic agent compared to other classes of antibiotics for the purpose of being used in livestock. This accounted for around 67% of the total sales. Additionally, penicillins and macrolides were commonly sold as well accounting for 12% and 8% of sales respectively (*FDA Reports Another Rise in Antibiotic Sales for Livestock*, 2020). Broilers have been recently used as a meat source and the world's largest broiler meat producers that account for 60% of the global broiler production include the United States, India, China, Brazil, Poland, Germany, the United Kingdom, Spain, and France (Zamai et al., 2016).

Antibiotics such as fluoroquinolones, macrolides, third-generation cephalosporins, and polymyxins are used in countries with large poultry production except for the ban of fluoroquinolone in the USA and cephalosporins in the European Union (Roth et al., 2019). Additionally, all countries have allowed the use of tetracyclines, sulfonamides, penicillins, and aminoglycosides in poultry. Representatives of these antibiotic classes have a high antibiotic-resistant *E. coli* rate that is more than 40% in all countries except for ampicillin in the USA (Fair & Tor, 2014). Moreover, there is a low resistance rate in the USA towards fluoroquinolones and quinolones which is less than 5% due to the fact that this drug was banned from being used in the USA. On the other hand, the average resistance rate of *E. coli* towards fluoroquinolone is more than 40% in other countries including Brazil, European Union, and China since this drug is approved for usage. Even though quinolones and fluoroquinolones have been prohibited from being used in the USA, this did not eliminate the presence of resistant pathogens to these agents (Roth et al., 2019).

Antibiotics are used in poultry to prevent and treat infections as well as promote growth (History, 2010). In 2006 and 2017, using antibiotics as growth promoters was banned in the European Union and the USA respectively. However, this application is currently allowed in China and Brazil (Roth et al., 2019). Antibiotics are used to treat and prevent infections in all poultry-producing countries. For instance, antibiotics can be used for the treatment of intestinal infections including colibacillosis and necrotic enteritis, as well as other infections caused by *Salmonella, E. coli,* as well as *Clostridium spp* (Agunos et al., 2012).

Antibiotic-resistant organisms are evolving and their pressure is being raised due to the usage of antibiotics in poultry farming (Diarra & Malouin, 2014). Since *E. coli* is a commensal bacterium that is usually found in animals and humans, monitoring of such bacterium will help in epidemiological surveillance. Additionally, it can be utilized as an early warning surveillance system in order to detect new trends of resistance in cattle as well as resistance spread to food derived from animals (Efsa-q- & Force, 2008). Since *E. coli* is a bacterium that is widely spread and can be used as a model to detect resistance trends, it is considered as an indicator bacterium for resistance to the communities of Gram-negative bacteria (Roth et al., 2019).

1.5 AMR identification and Lab Techniques

Multiple techniques are used for the monitoring of antimicrobial resistance (AMR). One of the crucial techniques is bacterial cultures which are utilized in disease investigations. Selective and differential media can be utilized to identify a certain bacterium (Frasao et al., 2017). It is important to differentiate species since it can be used as an important approach in treatment management (Kaakoush et al., 2015). Clinical and laboratory standards institute (CLSI) is a conventional approach used for AMR by providing antibiotic concentrations that can inhibit bacterial growth (Miller et al., 2020). Isolated bacteria can be subjected to many doses of antimicrobial drugs under specific conditions. After that their ability to inhibit bacterial growth will be evaluated. Disk diffusion, agar dilution, and broth dilution are common approaches for the assessment of cultured bacteria (Epsilometer test). The susceptibility of the bacterial isolates to each antimicrobial agent is identified followed by the interpretation of the results according to the manufacturer's criteria (Turnidge & Paterson, 2007). Additionally, a culture will result in the detection of isolates that can be used for molecular approaches. That will be utilized in diseases investigations. Molecular approaches can be helpful in monitoring AMR (Frasao et al., 2017). Such approaches can help in comparing relatedness among pathogens and isolates which is prominent in disease investigation (Anjum, 2015).

1.6 Typing of *E. coli*

Bacterial typing is a useful technique that can be used to detect epidemics and the dissemination of resistant pathogens since it identifies genetic differences among isolates of the same species (Foxman et al., 2005). Currently, multiple typing methods are available and each method has some benefits and drawbacks regarding discriminatory power and cost (Sabat et al., 2013). *E. coli* can be classified through different criteria such as phylogroups, multilocus variable number of tandem repeat analysis (MLVA), pulsed-field gel electrophoresis (PFGE), sequence typing, random amplified polymorphic DNA (RAPD), and serotyping. These methods can be beneficial tools in the epidemiological investigation and infection control of pathogenic *E. coli* (Nielsen et al., 2014).

1.7 Phylotyping of *E. coli*

E. coli can be classified into multiple phylogroups based on the Clermont method including (A, B1, B2, C, D, E, and F) (Herzer et al., 1990). Group B2, and D were previously reported as pathogenic phylogroups related to extraintestinal strains (Boyd & Hartl, 1998) and most of the commensal strains are classified into group A (Bingen et al., 1998).

1.8 MLST Typing

One of the commonly used typing methods in multiple laboratories is multi-locus sequence typing (MLST) (Maiden et al., 1998). MLST was first used in1998, and since then multiple criteria have been utilized for other species and are currently available in databases. The sequence type of each strain is determined based on the presence of seven housekeeping genes that codes for metabolic processes in *E. coli*. These genes include *adk*, *fumC, gyrB, icd, mdh*, *purA*, and *recA* (Wirth et al., 2006).

1.9 National Antimicrobial Resistance Monitoring System

The Nationwide Antibiotic Resistance Monitoring System (NARMS) was established in 1996 in the USA to track antimicrobial resistance in *Salmonella, E. coli*, and *Campylobacter*, as well as multiple foodborne bacteria, and it is considered as a national public health monitoring system (McDermott et al., 2016). The United States Department of Agriculture (USDA) reports the presence of antimicrobial resistance annually in *E. coli* isolates. This is done through tracking strains recovered from retail meats, humans, and food-producing animals (Roth et al., 2019a).

1.10 Prevention and Control of *E. coli*

Antibiotic resistance is posing a huge threat to healthcare systems around the globe as well as the transmission of resistant pathogens in bacteria (Protection et al., 2008). It is of high necessity to gain knowledge regarding the mechanisms, spread, and evolution of resistance as a result of the rapid dissemination of multi-drug resistant pathogens. Horizontal gene transfer is the main cause of the resistance mechanisms and the transfer of resistant genes among species and pathogenic bacteria. It was previously reported that antibiotic usage leads to selection for resistant pathogens as well as increasing gene exchange in hospitals, farms, and human communities (Lupo et al., 2012).

In this regard, the implementation of intervention measures from farm to table is required for any surveillance program to successfully control and reduce *E. coli* infections. It is of high necessity that Consumers provide an implementation of preparation intervention controls as well as food handling. Unfortunately, it has been reported that many individuals eat high-risk foods as well as the incorrect handling and storage of food items. Additionally, consumers ignore warnings regarding dangerous food items (Sanchez et al., 2002). For instance, ground beef should be cooked until it is no longer pink. Moreover, During slaughter and preparation, it is possible for excrement to lead to meat contamination. For that reason, all precautions and protective measures must be followed to avoid such problems. In addition to that, animal-derived meals must be cooked prior to consumption as well as personal hygiene (Pettan-Brewer et al., 2021).

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2. CHAPTER II: Phenotypic Characterization of antibiotic-resistant *E. coli* isolated from retail meats in North Carolina (2018-2019).

Presented here is a part of the manuscript under the title "Phenotypic and genotypic characterization of antimicrobial-resistant *Escherichia coli* isolated from multiple retail meat types in North Carolina (2018-2019) "submitted to the International Journal of Food Microbiology.

2.1 Abstract

Escherichia coli is a commensal bacterium that is normally present in the intestine of humans and animals. Even though some of the *E. coli* strains are safe, other strains can lead to serious infections. Pathogenic *E. coli* strains can be a burden to public health; however, they can be an extra burden when they are associated with antibiotic resistance. The aim of this study was to phenotypically characterize *E. coli* isolates recovered from retail meats in North Carolina from 2018 till 2019. One hundred and thirty-three isolates were subjected to a pre-selected panel of antibiotics from the National Antimicrobial Resistance Monitoring System (NARMS) through using broth microdilution method based on the minimum inhibitory concentration susceptibility testing. The isolates showed resistance towards aminoglycosides, sulfonamides, beta-lactams, macrolides, tetracyclines, phenicols, and fluoroquinolones. The highest prevalence of *E. coli* isolates was found in ground turkey (42.9%) and chicken (20.3 %), followed by ground beef (18.9%) and pork (18%). Additionally, all of the isolates were susceptible to meropenem. Both chicken and turkey meats were more resistant to all of the NARMS panel of tested agents than beef and pork meats. More than 30% of the turkey isolates

were multidrug-resistant (resistant to more than three drugs) in comparison to 22.2% of chicken, 8.4 % of pork, and 8 % of beef isolates.

2.2 Introduction

Although *Escherichia coli* is a commensal microorganism that is naturally present in the intestine of humans and animals, not all *E. coli* strains are innocuous because some can lead to threatening diseases in food animals, and humans (Naylor ID et al., 2019). For instance, some *E. coli* strains are responsible for disorders such as hemolytic uremic syndrome and hemorrhagic colitis which are considered to be alarming infections in humans (Ferens & Hovde, 2011). In order to treat such infections, antibiotics are frequently utilized and considered as important agents to conquer pathogenic *E. coli* strains (Mooljuntee et al., 2010). The efficacy of antibiotics has changed medicine and protected millions of people however, it is now in danger due to the global burden of resistant bacteria (Lee Ventola, 2015). It has been agreed upon worldwide that *E. coli* is a critical organism when it comes to antimicrobial resistance due to its ease in acquiring resistance as well as its regular presence in animal guts as a commensal bacterium (S. Zhao et al., 2012).

Many countries reported an increase in infections caused by *Escherichia coli* (Bonyadian et al., 2019). Additionally, antibiotic resistance is increasing, and resistant pathogens are leading to a pressing burden on public health (MAZUREK et al., 2013). One of the resistant pathogens is *E. coli* which is capable of disseminating and that is why it is a significant microorganism when it comes to antibiotic resistance (Delsol et al., 2010; Naylor ID et al., 2019).

Due to low economic incentives and difficult regulatory requirements, pharmaceutical companies are experiencing hardship regarding novel drug research. In addition to that, misusing and overusing antibiotics have been blamed for the antibiotic resistance phenomenon (Prestinaci et al., 2015). In this regard, many countries have established resistance monitoring systems to track antimicrobial resistance to protect humans and animals. In the United States of America, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 to track antimicrobial-resistant pathogens. NARMS has many roles when it comes to resistance and one of them is to track antibiotic-resistant *E. coli* obtained from various food sources (Karp et al., 2017). Previous studies reported that resistant pathogens from animal origins can cause infections in humans (Aarestrup et al., 2008). Food animals can be a source of resistant strains since the intensive use of antibiotics in food animal production is blamed for the resistance phenomenon. Moreover, the normal flora present in the intestine of food animals can be reservoirs for resistant determinants. These resistant genes can easily be transferred through mobile elements which aid in the dissemination of multidrug-resistant pathogens (Blake et al., 2003).

In this study, we phenotypically characterized 133 *E. coli* isolates retrieved from retail meats in North Carolina from 2018-to 2019. This was done through the broth microdilution analysis by using a preselected panel of 14 antimicrobials established by NARMS. To the best of our knowledge, this is the first study as part of the NARMs surveillance system.

2.3 Materials and Methods

2.3.1 Sampling of multiple retail meat types

From 2018 to 2019, 80 samples of various meat types were collected per month from retail stores in North Carolina, resulting in 1920 samples collected during the study period. These samples included 40 samples of chicken with bone-in or skin-on, 10 samples of ground pork chop, 20 samples of ground turkey, and 10 samples of ground beef. The meat was randomly selected and purchased based on geographically coded sites using zip codes representing each visited location. One hundred eighty-eight *E. coli* isolates were isolated among the collected samples (9.7%). As part of the NARMS program, 133 isolates were randomly selected. These isolates were further phenotypically and genotypically characterized and included in this study. The 133 isolates were obtained from ground turkey (n= 57), chicken (n= 27), ground beef (n= 25), and pork chop (n= 24).

2.3.2 Microbiological Analysis

Retail meats were cultured for *E. coli*. One intact piece of chicken or pork meat or 50 g of ground beef or turkey products were used. We placed each sample into a sterile plastic bag containing 250 mL buffered peptone water (ThermoFisher Scientific, Waltham, MA) followed by a vigorous shaking at 200 rpm for 15 minutes. The rinsate of each sample (50 mL) was transferred to a sterile flask and carefully mixed with 50 mL of double-strength MacConkey broth (ThermoFisher Scientific, Waltham, MA) and incubated at 35°C for 24 hours.

Ten microliters from each flask were subsequently streaked for isolation onto a MacConkey agar plate (ThermoFisher Scientific, Waltham, MA) followed by incubation for 24 hours at 35°C. Pink colonies consistent with *E. coli* morphology were selected; one individual colony was struck for isolation onto a blood agar plate. Indole and oxidase tests were performed and confirmed as *E. coli* by MALDI-TOF; all confirmed isolates were preserved in Brucella broth with 15% glycerol for cold storage at -80°C.

2.3.3 Phenotypic characterization through antibiotic susceptibility testing

The broth microdilution method was used for the phenotypic characterization of 133 *E. coli* isolates using the NARMS Sensititre panel (ThermoFisher Scientific, Waltham, MA) with human breakpoints interpreted per Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute Standards Development Policies and Process, 2013). The panel included: ceftriaxone (AXO; 0.25 - 64 µg/mL), azithromycin (AZI; 0.12–16 µg/mL), ampicillin (AMP; 1-32 µg/mL)), cefoxitin (FOX; 0.5-32 µg/mL)), ciprofloxacin (CIP; 0.015–4 µg/mL), nalidixic acid (NAL; 0.5–32 µg/mL), meropenem (MER; 0.06–4 µg/mL), gentamicin (GEN; 0.25–16 µg/mL), streptomycin (STR; 2-64 µg/ mL), chloramphenicol (CHL; 2-32 µg/ mL), tetracycline (TET; 4-32 µg/ mL), amoxicillin-clavulanic acid (AMC; 1/0.5–32/16 µg/mL), sulfisoxazole (FIS; 16-256 µg/ mL) and cotrimoxazole (COT; 0.12/2.38–4/76 µg/mL). If an isolate demonstrated resistance to at least three drugs or more antimicrobial classes, it was viewed as multidrug-resistant (MDR).

2.3.4 Statistical Analysis

Proportional comparisons were assessed using the Chi-square test for all isolates retrieved from different meat types when significant differences among samples existed. Moreover, the Kruskal Wallis test was conducted to compare samples from more than two groups. These analyses were done using R version 4.1 software. For each comparison, a p-value was considered to be statistically significant if it was less than 0.05.

2.4 Results

2.4.1 Antibiotic resistance Prevalence in *E. coli* from retail meats

From 2018 to 2019, 188 *E. coli* isolates were detected from 1,920 meat samples, for an overall prevalence of 9.8%. Out of the 188 isolates, 133 isolates were selected as representatives for evaluating phenotypic and genotypic antimicrobial resistance profiles. Ground turkey (n=57, 42.9%), chicken (n=27, 20.3%), ground beef (n=25, 18.8%) and pork chop (n=24, 18%) made up the distribution of sample types.

2.4.2 Phenotypic characterization through antimicrobial susceptibility

Fifty-five *E. coli* isolates (41.4%) were pan-susceptible to all of the antibiotics evaluated. There were 22.6% (30 of 133) *E. coli* isolates resistant to one antimicrobial agent, and 16.5% (22 of 133) showed resistance towards two antimicrobial agents (Table 1). Meat type significantly affected *E. coli* resistance profiles. Ground turkey isolates displayed the highest resistance level compared to other meat types ($p < 0.05$). The distribution of antimicrobial agent resistance by meat type is in Table 1. Twenty-five isolates (18.7%) were classified as MDR as they showed resistance towards three or more antimicrobial classes in the panel. Ground turkey had more multidrug-resistant isolates (n=16, 28%) than chicken (n=18.5%), beef (n=2, 8%), and pork (n=2, 8%). Additionally, one chicken isolate was multidrug-resistant to 6 antimicrobial classes including tetracycline, aminoglycoside, penicillin, folate pathway inhibitor, cephem, and β-Lactamase inhibitor combination. The most common resistance in *E. coli* isolates was towards tetracycline (48.9%), streptomycin (27.8%), ampicillin (23.3%), and sulfisoxazole (21.1%) (Table 2). All of the 133 *E. coli* isolates were susceptible to meropenem (Figure 1).

2.5 Discussion

Due to the intensive use of antibiotics in food animal production, antibiotic resistance was promoted, and resistant food-borne pathogens resulted in a burden to public health (Ma et al., 2021). In this regard, a monitoring system is necessary to track antimicrobial resistance dissemination. A surveillance program called NARMS was established in the USA in 1996 to track antibiotic resistance ubiquity in *E. coli* isolated from retail meats. The isolates were retrieved from multiple retail meat types in North Carolina from 2018 to 2019 through our study as a critical part of the surveillance of commensal and food-borne bacteria. This study on the phenotypic characterization of *E. coli* isolated from retail meats is considered to be the first phenotypic profiling regarding retail meats' *E. coli* isolates in North Carolina. In this study, we evaluated resistance in *E. coli* obtained from retail meat in North Carolina from 2018 to 2019. *Escherichia coli* prevalence, antimicrobial susceptibility, resistance genes, phylogroups, and sequence types were reported. We found that *E. coli* isolates from retail ground turkey were phenotypically more resistant to most of the antimicrobial agents ($p < 0.05$) than chicken, beef, and pork. Additionally, a higher number of resistant genes was identified in turkey isolates when compared to the other meat types ($p < 0.05$). Our results support those from multiple retail meat types previously reported from the United States (Johnson et al., 2005; C. Zhao et al., 2001). From 2001 to 2003, a survey was conducted on retail meats purchased from 10 retail stores that were included from the Minneapolis-St.Paul, MN, area. In that survey, the research group found a rate of contamination of 92% in poultry including chicken and turkey as well as 69% in beef and pork (Johnson et al., 2005). Another report regarding retail meats collected from four different states (Tennessee, Georgia, Oregon, and Maryland) in the United States from 2002 to 2008 yielded similar results, with contamination in poultry at around 80%, as well as 68%, and

44 % in beef and pork, respectively (S. Zhao et al., 2012). Other studies from the United States and multiple countries have reported varying rates of recovery. These studies have found *E. coli* to be a common contaminant of retail meat products. (Cook et al., 2009; Schroeder et al., 2003; C. Zhao et al., 2001).

Our study used whole-genome sequencing and broth microdilution for AMR characterization and relied on comparing phenotypic and genotypic results for *E. coli* isolates recovered from retail meats from 2018 to 2019. As a result, *E. coli* isolates showed frequent phenotypic resistance towards GEN, STR, TET, FIS, and AMP. Levels of antibiotic resistance varied and depended on the different sources of meat types of the isolates included in this study. The resistance rate of *E. coli* isolates recovered from ground turkey meats was higher than the rates found in chicken, beef, and pork isolates ($p < 0.05$). We observed 18.7% of the 133 isolates showed resistance to at least three or more drugs, and ground turkey had the highest number of MDR isolates ($p < 0.05$). Simultaneously, turkey isolates were more phenotypically resistant to AMP, CHL, TET, GEN, FIS, and STR than other meat isolates ($P < 0.05$). Although isolates from pork meat demonstrated lower phenotypic resistance than isolates from turkey, they generally had higher phenotypic resistance to FIS, STR, AMP, and CHL than beef isolates ($p < 0.05$). It has been hypothesized that higher resistance levels in turkey are partly due to modern production practices that rely on administering antimicrobials through water and feed to control and prevent diseases. In addition, selection pressure evolution regarding AMR stemmed from exposing large numbers of animals in a production facility to antimicrobials (Witte, 2000).

A small percentage of both ground turkey and pork isolates (3%) showed resistance towards chloramphenicol (CHL). These findings were consistent with a previous study that traced antibiotic-resistant *E. coli* in retail meats from 4 states in the USA (S. Zhao et al., 2012).

Chloramphenicol was banned in the 1980s from being used in the United States food animal production (Bischoff et al., 2002). Still, previous studies reported chloramphenicol resistance in previous studies in *E. coli* recovered from animal origins (Bischoff et al., 2002; S. Zhao et al., 2009). Moreover, florfenicol, an antibiotic related to CHL, was discovered in the 1980s and has been used in veterinary medicine in many countries (Ahmer et al., 2020). The *floR* resistance gene of the florfenicol drug confers a cross-resistance towards CHL (Bischoff et al., 2002).

2.6 Acknowledgment

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Table 2.1. Resistance patterns prevalence of *E. coli* recovered from multiple retail meat types.

Number of antimicrobials	**Number of resistant isolates (%)**	**Ground Turkey, n = 57(%)**	**Ground Beef, n = 25 (%)**	**Chicken, n = 27 (%)**	**Pork Chop, n = 24 (%)**
0	53(39.8)	11 (19.3)	18 (72)	12 (44.4)	12 (50)
1	30 (22.6)	16 (28.1)	4 (16)	6 (22.2)	4 (16.7)
2	22 (16.5)	12 (21.1)	0	3 (11.11)	7 (29.2)
3	9 (6.8)	5 (8.8)	2 (8)	2 (7.4)	0
4	9 (6.8)	6 (10.5)	0	2 (7.4)	1 (4.2)
5	5 (3.8)	5 (8.8)	0	0	0
6	2 (1.5)	1 (1.8)	0	0	1 (4.2)
7	2 (1.5)	1 (1.8)	0	1 (3.7)	0
8	1 (0.8)	0	0	1 (3.7)	0

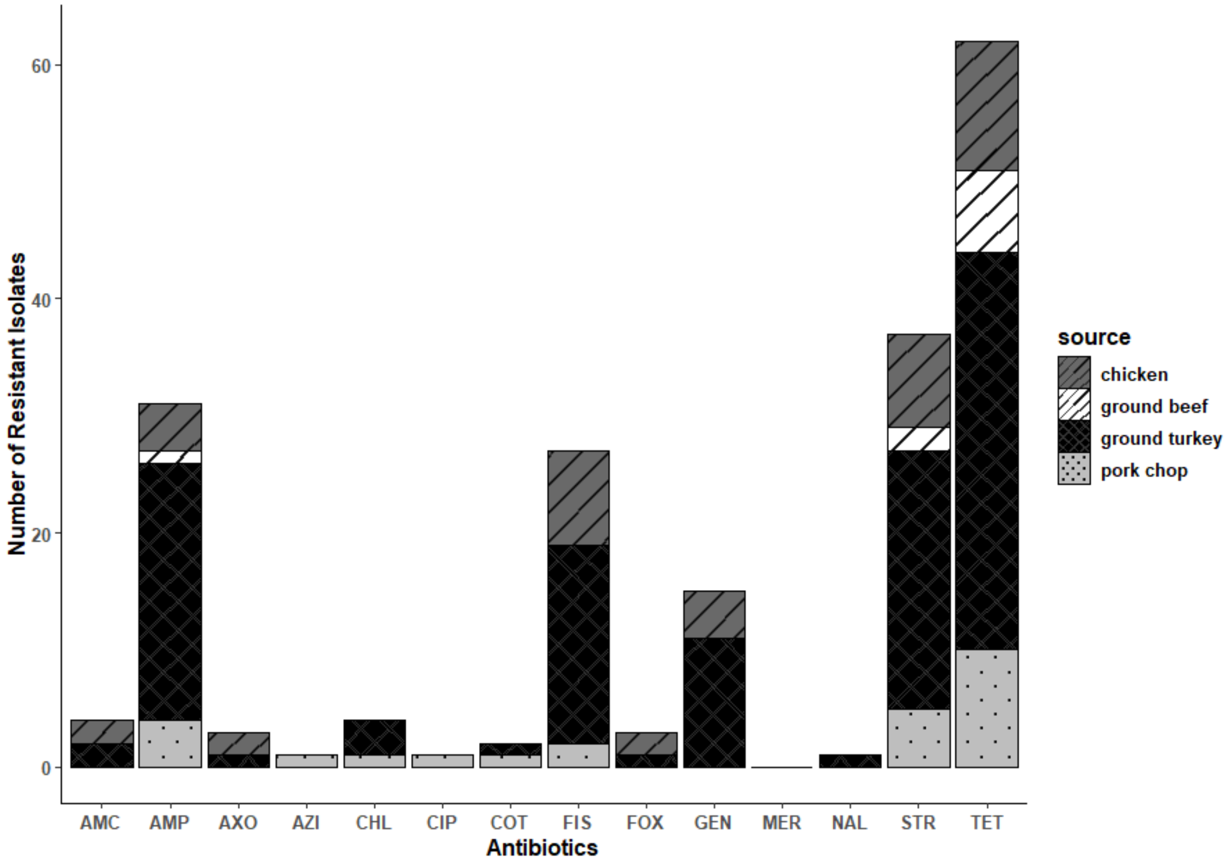

Fig 2.1. Antimicrobial resistance prevalence of *E.coli* isolates from different retail meat types purchased at North Carolina from 2018-2019. (AMP; Ampicillin), (AXO; ceftriaxone), (GEN; gentamicin), (STR; streptomycin), (TET; tetracycline), (COT; cotrimoxazole), (AZI; azithromycin), (CIP; ciprofloxacin), (NAL; nalidixic acid), (AMC: amoxicillin/clavulanic acid), (CHL; chloramphenicol), (FOX; cefoxitin), (FIS; sulfisoxazole).

Table 2.2. Antibiotic resistance prevalence and multiple resistances in *E. coli* recovered from different Retail Meat types sampled at North Carolina from 2018-2019. The preselected panel that was used included: (AMP; Ampicillin), (AXO; ceftriaxone), (GEN; gentamicin), (STR; streptomycin), (TET; tetracycline), (COT; cotrimoxazole), (AZI; azithromycin), (CIP; ciprofloxacin), (NAL; nalidixic acid), (AMC: amoxicillin/clavulanic acid), (CHL; chloramphenicol), (FOX; cefoxitin), (FIS; sulfisoxazole).

Antimicrobials	**Overall, n = 133 (%)**	**Ground Beef, n = 25 (%)**	**Ground Turkey, n= 57 (%)**	**Chicken, n = 27 (%)**	**Pork Chop, n=24 (%)**
AMC	4 (3)	0	2 (3.5)	2 (7.4)	0
AMP	31 (23.3)	1 (4)	22 (38.5)	4 (14.8)	4 (16.66)
FOX	3 (2.3)	0	1 (1.75)	2 (7.4)	0
AXO	3 (2.3)	0	1 (1.75)	2 (7.4)	0
CHL	4 (3)	0	3 (5.26)	0	1 (4.16)
GEN	15 (11.3)	0	11 (19.29)	4 (14.8)	0
STR	37 (27.8)	2 (8)	22 (38.59)	8 (29.6)	5 (20.8)
FIS	28 (21.1)	0	17 (29.8)	8 (29.6)	2 (8.3)
TET	65 (48.9)	7 (28)	34 (59.64)	11 (40.74)	10 (41.66)
COT	2 (1.5)	0	1 (1.75)	0	1 (4.16)

AZI	1 (0.8)	0	0	0	1 (4.16)
CIP	1 (0.8)	0	0	0	1 (4.16)
NAL	1 (0.8)	0	1 (1.75)	0	0

3. **CHAPTER III:** Genotypic characterization of antimicrobial-resistant *E. coli* isolates from retail meats in North Carolina (2018-2019).

Presented here is a part of the manuscript under the title “Phenotypic and genotypic characterization of antimicrobial-resistant *Escherichia coli* isolated from multiple retail meat types in North Carolina (2018-2019) “submitted to the International Journal of Food Microbiology

3.1 Abstract

Antimicrobial resistance characterization plays a critical role in yielding information regarding bacterial pathogenicity. In addition, such information can be used to provide suitable treatment against bacterial infections. In this report, we used whole-genome sequencing to sequence 133 *E. coli* isolates that were recovered from multiple retail meat types purchased from a myriad number of retail stores in North Carolina. Our sequences were screened for the detection of resistance genes. We observed a uniformity for each isolate regarding the relationship between phenotypic resistance and genotypic resistance. *Tet* and *aph* genes were the most prevalent among the identified resistant genes that result in the presence of resistance towards tetracycline and aminoglycoside antibiotics respectively. In silico screening resulted in discovering 128 sequence types (STs) as well as classifying our isolates into 7 phylogroups. Relationships between resistance and both phylotyping and sequence typing can lead to useful insights concerning the epidemiological track of resistance trends as well as providing effective interventions to handle antibiotic resistance.

3.2 Introduction

Resistance genes can be acquired, maintained, and transmitted by *E. coli* from other organisms in the environment (Puvača & Frutos, 2021). For that reason, *E. coli* is an important indicator when it comes to antibiotic resistance dissemination (Uddin, 2014). It has been proposed that *E. coli* strains recovered from animals have serious and threatening public health consequences. Some investigations have shown that *E. coli* isolated from animal origins are responsible for human infections and that they were found to be carrying the same resistance genes identified in a variety of bacterial species recovered from various animal origins (Mathew et al., 2009.; Poppe et al., 2005). In a previous report, it was found that the results concerning the ubiquity of antibiotic resistance in *E. coli* retrieved from chicken, turkey, and, swine were compatible with the resistance in *E. coli* isolates found in blood collected from humans (Vieira et al., 2011).

According to estimates, approximately 700,000 people die every year because of pathogens acquiring resistance towards antibiotics around the world. Additionally, it was estimated that the antibiotic resistance phenomenon will claim at least 10 million lives annually by the year 2050 unless effective mitigation measures are implemented (Barber & Sutherland, 2017; Guo et al., 2019). Due to low economic incentives and difficult regulatory requirements, pharmaceutical companies are experiencing hardship regarding novel drug research. In addition to that, misusing and overusing antibiotics have been blamed for the antibiotic resistance phenomenon (Prestinaci et al., 2015). In this regard, many countries have established resistance monitoring systems to track antimicrobial resistance to protect humans and animals. In the United States of America, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 to track antimicrobial-resistant pathogens. NARMS has many roles when it comes to resistance and one of them is to track antibiotic-resistant *E. coli* obtained from various food sources (Karp et al., 2017).

E. coli is considered to be a leading cause of foodborne diseases in the United States (Mead et al., 1999). Bacteria can be described by using genotypic characterizations of antibiotic resistance genes. Genotypic characterization can be utilized as a way to investigate the distribution of resistance genes (C. D. Miranda et al., 2003). There are multiple approaches utilized for the detection of antimicrobial resistance genes including PCR arrays, DNA hybridization, and sequencing (Chen et al., 2004; C. D. Miranda et al., 2003). Moreover, it has been reported that *E. coli* is one of the primary reasons for urinary tract infections and septicemia. In addition to that, the increased dissemination of antibiotic-resistant *E. coli* represents a burden on the health care systems all over the world because there is a difficulty in treating such infections (Gross, 2013; Sengupta et al., 2013).

The versatile ability of the microbial community to adapt to environmental challenges led to the current antimicrobial resistance (AMR) crisis. This adaptability will continue to occur, and AMR will increase as long as the microbial populations are subjected to intense selective pressure (Michael et al., 2014). Medical professionals are currently expressing grave alarm about a return to the pre-antibiotic era (Baral & Mozafari, 2020). Fortunately, upon analyzing bacterial genomes, around 20,000 resistance genes were discovered, nevertheless, it was reported that functional resistance genes are less in number (Aslam et al., 2018; Sengupta et al., 2013). Recently, there have been breakthroughs in genomics and sequencing as well as decreased sequencing costs (Köser et al., 2014). As a result, whole-genome sequencing can be utilized as a way of analysis to predict antimicrobial resistance and antimicrobial susceptibility which can be done through the detection of resistance genes present. Hence, it has a lot of potential in terms of epidemiological typing, and resistance profiling (Espedido et al., 2015).

Molecular typing methods are necessary for the investigation of outbreaks, surveillance systems, and the tracking of resistance trends in bacterial pathogens (Fladberg et al., 2017). It is of great necessity to the entire world that genotypes should be monitored in order to help in the management of antibiotic resistance as well as limit the spread of resistant bacteria (Mathers et al., 2015a). One of the common typing methods is multilocus sequence typing (MLST) as it is advantageous for being an easy way to generate data. However, MLST does not provide enough resolution in case of an outbreak investigation (Fladberg et al., 2017).

According to the O'Neil study, antibiotic resistance results in the death of 50,000 people annually and that is only in the USA and Europe. It was also estimated that by the year 2050, around 10,000,000 people will die every year due to resistance (Barber & Sutherland, 2017). Antibiotic resistance is a phenomenal trend that must be monitored globally to limit its dissemination. One way to do that is by monitoring the genotypic profiles of resistant pathogens (Mathers et al., 2015b). In this study, we genotypically characterized 133 *E. coli* isolates retrieved from retail meats in North Carolina for the entire period of two years from 2018 to 2019.

3.3 Materials and Methods

3.3.1 DNA isolation and sequencing

Following the manufacturer's instructions, DNA was purified from each *E. coli* isolate using the DNAeasy purification kit (Qiagen, Venlo, Netherlands). Then, Qubit 4.0 fluorometer was used for DNA quantification (ThermoFisher Scientific, Waltham, MA). The Nextera DNA kit was used to prepare DNA libraries (Illumina, San Diego, CA). This was done through the utilization of 300 bp paired-end reads (MiSeq reagent kit, v3). Subsequently, the samples were submitted to the National Center for Biotechnology Information GenomeTrakr database (Accession: PRJNA293225).

3.3.2 Whole-genome sequence analysis

SPAdes assembler v.3.13.1 was used to perform denovo assembly on the Illumina paired end reads for each isolate (Bankevich et al., 2012). In silico identification of resistant genes and β-lactamases took place using ABRicate (https://github.com/tseemann/abricate) program including Resfinder (Zankari et al., 2012), and CARD (Jia et al., 2017). A 95%-100% identity was used to match each identified gene to the annotated resistance genes (Zankari et al., 2012). In addition, in silico analysis of the isolates through multilocus sequence typing (MLST) was done using a previously described criterion. This scheme assigns sequence types (STs) to each isolate based on the allelic variation among seven housekeeping genes: *purA, fumC, icd, adk, mdh, gyrB,* and *recA* (Wirth et al., 2006). MLST finder 2.0 was used to assign a sequence type to each isolate with a match of 100% against known alleles (Larsen et al., 2012). Moreover, a previously described technique (Clermont et al., 2015) was utilized to assign all of the 133 *E. coli* isolates to one of the phylogroups including A, B1, B2, C, D, E, or F. According to Clermont et al., this technique results in the detection of four genes, including *chuA, arpA, TspE4,* and *yjaA.* The presence or absence of these four genes would assign an *E. coli* isolate to a specific phylogroup (Clermont et al., 2015). An in silico typing method was used to identify phylogroups. The web interface can be accessed through http://clermontyping.iame-research.center/ (Beghain et al., 2018)

3.4 Results

3.4.1 Genotypic characterization through whole-genome sequencing

All 133 isolates were characterized by whole-genome sequencing. Genomic analysis revealed that resistance genes related to aminoglycoside antibiotic were discovered in 60% of the isolates and included; *aph(3'')-Ib* (16.5%), *aph(6)-Id* (15.8%), *aph(3')-Ia* (8.3%), *aac(3)VIa (7.5%)* *aph(4)-Ia* (3.8%), *aac(3)-IV* (3.8%), *aac(3)-IId* (3%), and *aad2* (1.5%). In 16.7% of the isolates, β-lactam resistance genes were found, including *TEM-1* (11.3%), *TEM-12* (0.8%), *TEM-141* (0.8%), and *TEM-150* (3.8%). In addition to that, *qnrB*, a fluoroquinolone-resistant gene was found in one isolate from pork (0.8%). Moreover, resistant transferable genes related to sulphonamide antibiotics were identified in 22.6% of the isolates including *sulI, sulII, sulIII,* as well as other folate pathway inhibitors including *dfrA12*(2.3%) and *dfrA1*(0.8%). Additionally, florfenicol resistance gene *floR* was identified in 3 isolates (2.3%). Furthermore, the most common tetracycline resistance genes that were identified included *tetB* (25.6%), *tetA* (24%), and *tetC* (0.8%). A macrolide resistance gene, *mphA*, was discovered in one isolate from pork (0.8%) (Table 3).

3.4.2 Phenotypic and genotypic comparison of *E. coli* isolates

Phenotypic and genotypic resistance patterns were compared across the four meat types using the Kruskal Wallis test. Multi-drug resistant isolates were more frequent in turkey (28%, n = 16/57) compared to other meat types ($p < 0.05$). That showed similarity to the genotypic profile since most of the turkey isolates carried resistant genes to three antimicrobial classes or more (Table 3). Isolates from turkey showed higher phenotypic resistance to AMP, CHL, GEN, STR, FIS, TET, and NAL ($p < 0.05$) than the other meat types. The phenotypic resistance of turkey isolates was similar to the genotypic profile since they carried the highest number of resistant genes among all of the meat types ($p < 0.05$) including folate pathway inhibitor-resistant genes (*sulI*, *sulII*, *dfrA*, and *dfrA12*), tetracycline-resistant genes (*TetA*, and *TetB*), beta-lactam resistant genes (*TEM-1*, *TEM-141*, *TEM-150*, and *TEM-141*), aminoglycoside resistant genes (*aph* and *aac* genes) (Table 3). On the contrary, chicken isolates did not present higher phenotypic or genotypic resistance compared to pork and beef meats ($p = 0.2$). Moreover, pork isolates were more phenotypically resistant than beef for certain antibiotics including AMC, AMP, FOX, AXO, GEN, STR, FIS, and TET ($p < 0.05$). Additionally, pork isolates had more resistant genes than beef isolates including *sulI, sulIII*, *dfrA12, TetB, qnrB, TEM-1, aph(6)-Id*, *aph(3'')-Ib*, *aad2*, *aphA*, and *floR* ($p = 0.004$) (Table 3).

3.4.3 Phylogenetic analysis

The isolates were grouped into seven different phylogenetic groups, with B1 (44.4%) having the highest prevalence, followed by A (29.3%), B2 (10.5%), D (5.3%), F (4.5%), E (2.3%), and C (1.5%). This study also revealed that 2.3% of the isolates were unclassified (Table 4).

3.4.4 Multilocus sequence typing (MLST) analysis

The 133 isolates in this study belonged to 128 different sequence types (STs). Sixteen different STs were observed multiple times, including ST428 (n=4), ST58 (n=7), ST155 (n=3), ST602 (n=3), ST2175 (n=4), ST101 (n=3), ST847 (n=2), ST1463 (n=2), ST117 (n=3), ST10 (n=16), ST540 (n=2), ST216 (n=2), ST616 (n=2), ST355 (n=2) as shown in (Figure 2). The remaining sequence types were only observed once, and ST131 was one of them.

3.5 Discussion

Antimicrobial resistance and resistant pathogens represent a global concern to public health since resistant determinants can easily be transmitted among pathogens (Balbin et al., 2020). For that reason, the National Antimicrobial Resistance Monitoring system has long been aiming at tracking resistance and resistant genes through the phenotypic and genotypic profiling of resistant pathogens. Our data show that there is a small percentage of both ground turkey and pork isolates (3%) showed resistance towards chloramphenicol (CHL). Furthermore, CHL resistance can result from the co-selection of florfenicol (S. Zhao et al., 2012). Our data show that the florfenicol resistant gene (*floR*) in ground turkey and pork (2.3%) isolates is consistent with the phenotypic expression of chloramphenicol resistance. In addition, ciprofloxacin resistance (CIP), a fluoroquinolone drug, was discovered in a single isolate from pork. Fluoroquinolone is an antibiotic that can be utilized in the management of *Salmonella* infections in humans. This drug was banned in 2005 in the USA by the Food and Drug Administration (FDA) from being used in poultry to minimize the existence of fluoroquinolone-resistant *Campylobacter* (Price et al., 2007). Although fluoroquinolone is still used in swine and cattle to treat respiratory diseases (Extralabel Use and Antimicrobials | FDA, 2021), its resistance is a concern to public health that can result in failure of treatment as well as using other drugs with more side effects (Tyson et al., 2019).

Carbapenems, including meropenem (MER) are used to manage MDR infections and they are considered last-resort antibiotics (Sekyere, 2016). No resistance towards meropenem was observed, indicating the absence of carbapenemase in these *E. coli* isolates. That is consistent with NARMS reports (NARMS-2011-Executive-Report---Full-Report, 2011; NARMS Now:Integrated Data|FDA, 2022). Additionally, all 133 isolates showed low resistance towards COT and NAL, which is consistent with the findings of previous NARMS reports (NARMS Now: Integrated Data | FDA, 2022.; Sheikh et al., 2012).

The sequence identified resistance genes correlated with the phenotypic results based on the NARMS panel. However, there is a possibility that resistance genes exist without phenotypic antibiotic resistance being expressed (Rosengren et al., 2009). *TEM* is a β-lactamase gene that was frequently found in the isolates of this study (16.5%). Consequently, *E. coli* derived from poultry carried more beta-lactamase genes (n = 16, 19%) than *E. coli* from both pork (n=4, 16.6%) and beef (n=2, 8%) (Table3). It was also discovered that a high number of the isolates showed phenotypic resistance towards beta-lactam antibiotics which makes the previous finding plausible and implies that related resistance genes are present. Moreover, resistance genes including *tetB*, *sul2*, and *aph(3'')-1b* were the most common among the 14 identified non-beta-lactam antibiotic resistance genes (25.6% , 12.8% and 16.5%, respectively). Tetracycline is used to treat poultry infections; it is not expensive and does not lead to serious side effects (Chopra & Roberts, 2001; Miranda et al., 2009). Despite the critical role of tetracyclines in medicine regarding both humans and animals, the evolution of antibiotic resistance has narrowed their efficacy in treating and managing diseases (Chopra & Roberts, 2001). Koo and Woo discovered that most meat and meat product isolates (98.3%) carried not less than a single *tet* gene. They also reported that *tetA*, and *tetB* had the highest frequencies (52.4% and 41.3%, respectively), with *tetC* at the lowest frequency of 1.7% (Koo & Woo, 2011). These results are similar to the findings of this study as *tetC* (0.8%) was the least prevalent among the tetracycline genes, whereas *tetA* and *tetB* had the highest frequency. However, *tetB* (25.6%) had a higher prevalence than *tetA* (24%).

Furthermore, the *qnr* genes in *Enterobacteriaceae* confer poor resistance to quinolones. Previous reports demonstrate *E. coli* resistance to ciprofloxacin (CIP); additional reports document CIP genes' detection and expression, including *qnrA, qnrB,* and *qnrS* (Hamed et al., 2018; Mahmoud et al., 2020). Interestingly, a *qnrB* gene was found in a single isolate from pork. Since quinolone-resistant *E. coli* can be acquired through the food chain, it is highly emphasized that the dissemination of the *qnr* gene from *E. coli* recovered from animals should not be dismissed (Yue et al., 2008). Additionally, macrolide resistance (0.8%) was observed in one pork isolate (identification of the *mphA* gene), consistent with the phenotypic profile of resistance towards azithromycin. This is also compatible with the findings of NARMS data, as minimum resistance towards azithromycin was reported (0.1%-0.4%) from 2011 till 2021 (NARMS Now: Integrated Data | FDA,2022).

The Clermont phylotyping method has been used for detecting *E. coli* phylogroups (M. Wang et al., 2021). Based on the findings of this study, applying the Clermont method divided all of the *133* isolates into seven phylogroups including A, B1, B2, C, D, E, or F. this study's results showed that groups A and B1 appeared more frequently compared to the other phylogroups. The relationship between antibiotic resistance and phylogroups revealed that all

E. coli isolates from group B1 showed antibiotic resistance to all agents tested, particularly tetracycline and streptomycin; no resistance towards ciprofloxacin and nalidixic acid was observed. A small percent of group A isolates had resistance towards ciprofloxacin (n=1, 2.5%), and nalidixic acid (n=1, 2.5%). Phylogroups including B2, C, D, E, and F had lower prevalence among the isolates. Previous reports indicated that group F is commonly identified in swine and cattle (Abraham et al., 2015; Ewers et al., 2014). An earlier report found that the majority of extraintestinal pathogenic *E. coli* (ExPEC) isolates that cause urinary tract infections (UTIs) included in that study belonged to phylogroups B2 and D. (Rodriguez-Siek et al., 2005). Correspondingly, establishing the ubiquity of each phylogroup in each retail meat and assessing resistance in those phylogroups may be more helpful in determining suitable antibiotic treatment and a better understanding of the epidemiology of pathogenic bacteria (Bakhtiari et al., 2020).

Furthermore, ST10 and ST58 were the most prevalent STs among the 133 isolates, accounting for 13% and 5.3% of isolates, respectively. ST10 was observed more frequently in poultry isolates than in the other meat types. A study conducted in the Netherlands reported that ST10 of ESBL *E. coli* was observed in chickens. Another Canadian study discovered MDR ST10 isolates in retail chicken and pork meat (Manges & Johnson, 2012). Moreover, ST58 was the most common clonal type in a study conducted in Korea (Kim et al., 2020). ST58, described as a major vector of extended-spectrum beta-lactamases (ESBL) genes, is globally disseminated(van Hoek et al., 2018). In addition, a single chicken isolate carrying ST131 was found among the 133 isolates. A study conducted in the USA reported that ST131 was the causative agent of many antimicrobial-resistant *E. coli* infections in 2007, posing a severe public health threat (Johnson et al., 2010).

In conclusion, *E. coli* isolated from retail turkey meat had higher antibiotic resistance than chicken, ground beef, and pork. The overall prevalence of resistance was consistent with previous reports from the U.S. Ongoing phenotypic and genotypic surveillance of antimicrobial resistance and monitoring the potential consumer exposure to resistant strains is critical for continued public health.

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Table 3.1 Antibiotic-resistant genes identified in *E. coli* recovered from Retail Meats.

Resistance Genes	**Overall, n = 133 (%)**	**Ground Beef, n = 25 (%)**	**Ground Turkey, n = 57 (%)**	**Chicken, n = 27 (%)**	**Pork Chop, n = 24 (%)**
sulI	11 (8.3)	0	6 (10.5)	4 (14.8)	1 (4.2)
sulII	17 (12.8)	1 (4)	10 (17.5)	5 (18.5)	1 (4.2)
sulIII	2 (1.5)	0	1 (1.8)	0	1 (4.2)
tetA	32 (24)	4 (16)	19 (33.3)	6 (22.2)	3 (12.5)
tetB	34 (25.6)	3 (12)	18 (31.6)	4 (14.8)	9 (37.5)
tetC	1 (0.8)	1 (4)	0	0	0
qnrB	1 (0.8)	0	0	0	1 (4.2)
TEM-1	15 (11.3)	2 (8)	9 (15.8)	0	4 (16.7)
TEM-12	1 (0.8)	0	0	1 (3.7)	0
TEM-141	1 (0.8)	0	1 (1.8)	0	0
TEM-150	5 (3.8)	0	4 (7)	1 (3.7)	0
aph(6)-Id	21 (15.8)	2 (8)	13 (22.8)	3 (11.11)	3 (12.5)
aph(4)-Ia	5 (3.8)	0	5 (8.8)	0	0
aph(3')-Ia	11 (8.3)	1 (4)	7 (12.3)	3 (11.11)	0
aph(3'')-Ib	22 (16.5)	2 (8)	13 (22.8)	3 (11.11)	4 (16.7)
aac(3)IV	5 (3.8)	0	5 (8.8)	0	0
aac(3)-IId	4 (3)	1 (4)	3 (5.3)	0	0
aac(3)-VIa	10 (7.5)	0	6 (10.5)	4 (14.8)	0

Table 3.2 Different phylogroups identified in *E. coli* isolated from four retail meat types in North Carolina from 2018-2019.

Phylogroup	**Overall, n = 133 (%)**	**Ground Beef, n = 25 (%)**	**Ground Turkey, n = 57 (%)**	**Chicken, n = 27 (%)**	**Pork Chop, n = 24 (%)**
A	39 (29.3)	5 (20)	15 (26.3)	5 (18.5)	14 (58.3)
B1	59 (44.4)	17 (68)	27 (47.4)	6 (22.2)	9 (37.5)
B2	14 (10.5)	0	11 (19.3)	3 (11.11)	0
C	2 (1.5)	0	1 (1.8)	1 (3.7)	0
D	7 (5.3)	0	3 (5.3)	3 (11.11)	1 (4.2)
E	3 (2.3)	2 (8)	0	1 (3.7)	0
F	6 (4.5)	0	1 (1.8)	5 (18.5)	0
Unclassified	3 (2.3)	0	2 (3.5)	1 (3.7)	0

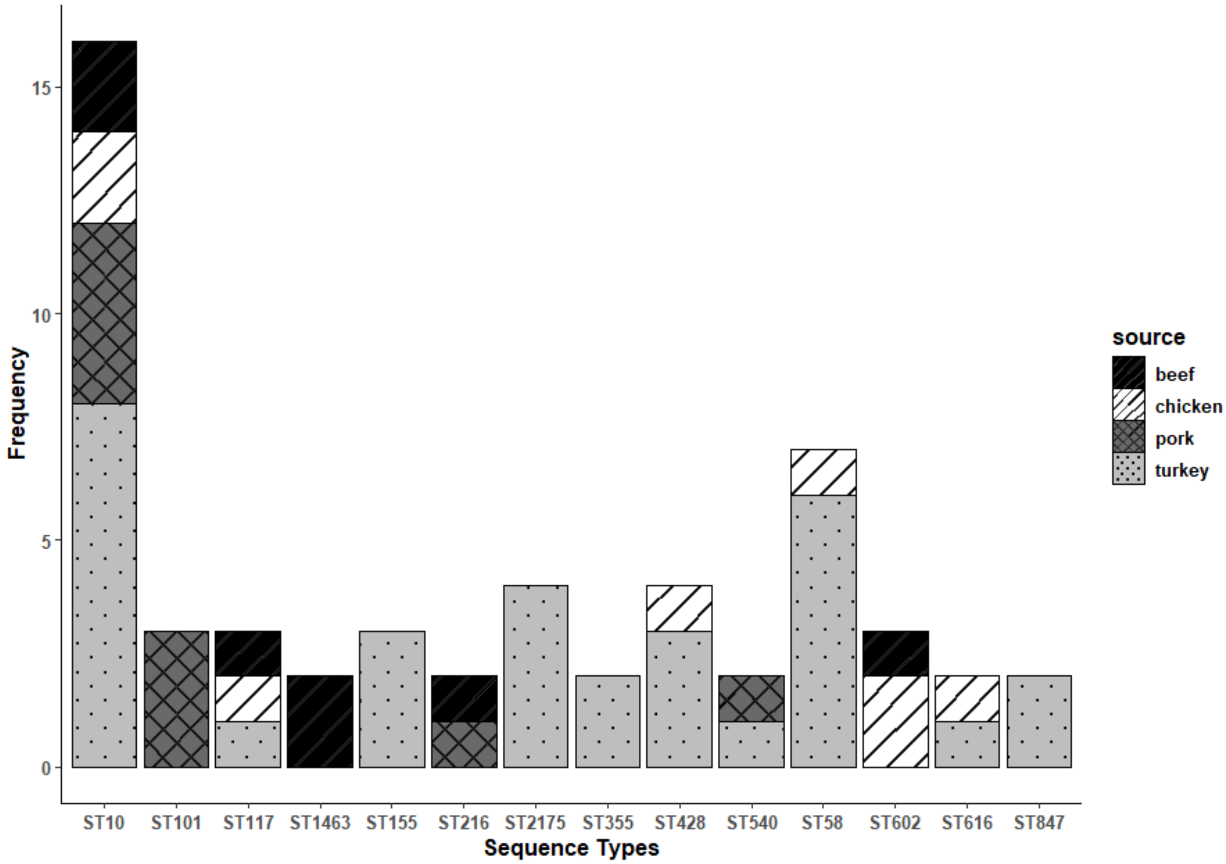

Fig.3.1. Prevalent sequence types identified in *E. coli* isolates recovered from four retail meat types in North Carolina from 2018-2019.