RESEARCH ARTICLE

Characteristics of antimicrobial resistance in *Escherichia coli* isolated from retail meat products in North Carolina

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Abstract

Background

Escherichia coli is commonly used as an indicator for antimicrobial resistance (AMR) in food, animal, environment, and human surveillance systems. Our study aimed to characterize AMR in *E. coli* isolated from retail meat purchased from grocery stores in North Carolina, USA as part of the National Antimicrobial Resistance Monitoring System (NARMS).

Materials and methods

Retail chicken (breast, n = 96; giblets, n = 24), turkey (n = 96), and pork (n = 96) products were purchased monthly from different counties in North Carolina during 2022. Label claims on packages regarding antibiotic use were recorded at collection. *E. coli* was isolated from meat samples using culture-based methods and isolates were characterized for antimicrobial resistance using whole genome sequencing. Multi-locus sequence typing, phylogroups, and a single nucleotide polymorphism (SNP)-based maximum-likelihood phylogenic tree was generated. Data were analyzed statistically to determine differences between antibiotic use claims and meat type.

Results

Of 312 retail meat samples, 138 (44.2%) were positive for *E. coli*, with turkey (78/138; 56.5%) demonstrating the highest prevalence. Prevalence was lower in chicken (41/138; 29.7%) and pork (19/138;13.8%). Quality sequence data was available from 84.8% (117/138) of the *E. coli* isolates, which included 72 (61.5%) from turkey, 27 (23.1%) from chicken breast, and 18 (15.4%) from pork. Genes associated with AMR were detected in 77.8% (91/117) of the isolates and 35.9% (42/117) were defined as multidrug resistant (MDR: being resistant to ≥3 distinct classes of antimicrobials). Commonly observed AMR genes included *tetB* (35%), *tetA* (24.8%), *aph(3'')-lb* (24.8%), and *bla*TEM-1 (20.5%), the majority of which originated from turkey isolates. Antibiotics use claims had no statistical effect on MDR *E. coli* isolates from the different meat types (X^2 = 2.21, p = 0.33). MDR was observed in

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isolates from meat products with labels indicating "no claims" (n = 29; 69%), "no antibiotics ever" (n = 9; 21.4%), and "organic" (n = 4; 9.5%). Thirty-four different replicon types were observed. AMR genes were carried on plasmids in 17 *E. coli* isolates, of which 15 (88.2%) were from turkey and two (11.8%) from chicken. Known sequence types (STs) were described for 81 *E. coli* isolates, with ST117 (8.5%), ST297 (5.1%), and ST58 (3.4%) being the most prevalent across retail meat types. The most prevalent phylogroups were B1 (29.1%) and A (28.2%). Five clonal patterns were detected among isolates.

Conclusions

E. coli prevalence and the presence of AMR and MDR were highest in turkey retail meat. The lack of an association between MDR *E. coli* in retail meat and antibiotic use claim, including those with no indication of antimicrobial use, suggests that additional research is required to understand the origin of resistance. The presence of ST117, an emerging human pathogen, warrants further surveillance. The isolates were distinctly diverse suggesting an instability in population dynamics.

Introduction

Surveillance of antimicrobial resistance (AMR) along the one-heath continuum, including in the food chain, is necessary to reduce the impact of resistant bacteria on health and prioritize policies and areas of intervention. The value and importance of one-health surveillance systems have been well described [1]. *Escherichia coli*, a gram-negative bacteria common to the human and animal gastrointestinal tract, is often used as an indicator organism in AMR surveillance systems [2]. Studies have shown that food products are potential reservoirs of pathogenic *E. coli* for humans [3, 4]. Most foodborne outbreaks in humans caused by *E. coli* have been associated with the consumption of contaminated food products of animal origin or contaminated with animal feces [5]; additionally, the role of these organisms in transmission of AMR to naturally-occurring human strains remains unknown and a concern. *E. coli* have flexible fitness mechanisms, making them easily adaptable to environmental conditions [6]. They can persist on surfaces for long periods of time and can be isolated from ready-to-eat food products [7].

To track AMR in food products of animal origin in the United States, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996. The NARMS program which is operated by the Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) routinely conducts surveillance of foodborne pathogens in humans, retail meat products and food animals respectively. *E. coli* is one of the indicator bacteria identified for AMR surveillance in NARMS [8]. The NARMS program contributes to the promotion and protection of public health by disseminating knowledge regarding new bacterial resistance, the distinction between resistant and susceptible illnesses, and the effects of treatments meant to stop the spread of resistance [9]. Since its inception, the NARMS program has been solely responsible for continued tracking of antimicrobial resistance among human and animal related populations through the food supply.

The use of antimicrobials in any setting has potential implications on the development and maintenance of AMR. In the United States, retail food products including meat have been marketed with antibiotic use claims since the mid-2000s [10]. There are public concerns that

the use of antimicrobials in food producing animals may affect the efficacy of similar drugs in human medicine especially through selection pressure of resistant bacteria and their ability to be transferred to humans through the food chain [11, 12]. The scientific evidence to support the risk of food products in disseminating AMR bacteria, including *E. coli*, is less clear. Studies have shown that resistance genes in bacteria such as *E. coli* can be transmitted at the human-animal-environment interface [13, 14]. Increasingly, modern molecular methods such as whole-genome sequencing (WGS) have been used to detect and trace the presence of resistance genes, mobile genetic elements and plasmids in *E. coli*, including from retail meat [15]. The NARMS surveillance program uses a WGS-based method for evaluating AMR in *E. coli* isolates from retail meat products.

Historically, the most prevalent AMR genes detected in *E. coli* from retail meat from the NARMS surveillance program include genes associated with erythromycin (*mph*(A)), tetracycline (*tet*(A), *tet*(B) and *tet*(C)), sulfonamide (*sul1* and *sul2*) and plasmid mediated quinolone (*qnr; gyr* or *par* mutations) resistance [16–18]. Recent analysis of the NARMS Genome Trackr database showed that common phylogroups detected in *E. coli* recovered from retail meat were A, B1, B2, C, Clade I, D, E, F, and G [18]. Previous NARMS studies have reported *E. coli* prevalence of 47.5% in all retail meat products, with higher prevalence (90.7%) reported in turkey products [17, 19]. While one study reports more than 50% of the isolates demonstrated multidrug resistance (MDR), which showed turkey meat-derived *E. coli* isolates with the highest resistance other studies have reported a lower MDR prevalence of 14.3% [17, 19].

Here, we provide updated prevalence estimates and characterization of the common AMR mechanisms and phylogeny characteristics of *E. coli* isolated from retail meat with different antimicrobial use claims including chicken breasts, pork chops and ground turkey purchased from retail grocery stores in North Carolina, USA.

Materials and methods

Retail meat sampling

Between January and December 2022, 312 meat products comprised of retail chicken cuts (breast; bone-in/skin-on; n = 96), ground turkey (n = 96), pork chop (n = 96) and chicken giblets (liver, gizzard, or heart; n = 24) were purchased from grocery supermarkets across five municipalities and eight counties in North Carolina in accordance with NARMS project protocol [20]. Monthly, 26 samples were randomly selected and purchased from grocery stores using zip codes that geographically represented each location based on US FDA assignment. At each sampling, eight chicken breast, eight pork chop, eight ground turkey and one package each of chicken heart, liver or gizzards were purchased.

As per the NARMS protocol for 2022, the demographic information collected for each purchased meat sample included antibiotic use claims apparent on product label, store name, store location, brand name, sell-by-date, purchase date, lab processing date and season of retail meat purchase (winter, spring, summer, and fall). Winter is considered December, January, and February; spring is March through May; summer is June through August; and fall is September through November. The samples were kept on ice in an isothermal container, during transportation from the grocery stores to the laboratory. On arrival at the lab, samples were refrigerated at 4˚C and processed within 96 hours for detection of multiple bacteria, including *E. coli*.

Bacterial culture

We followed the provided NARMS 2022 retail meat surveillance laboratory protocol; briefly, this began with 50g of each sample aseptically cut into a stomacher bag where 250 ml of Buffer

Peptone Water (Thermo Fisher Scientific, Waltham, MA) was added, and the sample was homogenized on a shaker at 200rpm for 15 mins. After mixing, the sample was placed into a sterile, plastic container (Fisherbrand™) with 50 ml of double strength (2X) MacConkey broth (Thermo Fisher Scientific, Waltham, MA), mixed well and incubated at 35˚C for 24h. Following incubation, a 10ul loopful of enrichment broth was plated onto MacConkey agar (BD BBL™, Sparks, MD) and incubated at 35˚C for 24h. One colony demonstrating typical *E. coli* phenotypic morphology (pink, round) was picked from each plate and streaked for isolation onto blood agar plates (Thermo Fisher Scientific, Lenexa, KS) which were incubated at 35˚C for 18-24h. Indole (BD BBL™, Sparks, MD) and oxidase (BD BBL™, Sparks, MD) quick tests were performed on suspected *E. coli* colonies; indole-positive, oxidase-negative colonies were confirmed to be *E. coli* by matrix-assisted laser desorption/ionization-time of flight (MALDI TOF; BioMerieux). All confirmed *E. coli* isolates were saved in cryovials containing *Brucella* broth (Thermo Fisher Scientific, Lenexa, KS) with 15% glycerol and subsequently stored at -80˚C for sequencing analysis.

DNA extraction and whole genome sequencing

DNA was extracted from each *E. coli* isolate using a modified version of the Qiagen DNeasy PowerLyzer microbial kit (Qiagen, Hilden, Germany). Using a 10µl loop, four passes of cells were added to 300µl of PowerBead solution in a 1.5 ml microcentrifuge tube. Next, 50µl of solution-SL was added to the PowerBead tubes which were immediately vortexed for 5 seconds to re-suspend. The bacterial suspension (300µl) was transferred to the PowerBead tubes which were placed in a bead mill (speed = 6; time = 1min) to homogenize. The PowerBead tubes were centrifuged (30secs at 17,000xg), and the supernatant was transferred to new 1.5 ml microcentrifuge tube (Qiagen, Hilden, Germany). Afterwards 100µl of solution-IRS was added to the supernatant and vortexed briefly, followed by a 5 min incubation at 4˚C. After incubation the tubes were centrifuged, and the supernatant again transferred to a new 1.5 ml microcentrifuge tube each containing 900µl of solution-SB. The tubes were vortexed and 650µl of supernatant transferred to the spin column for spin washing (3 washes) and final elution. The Nanodrop 2000 Spectrophotometer was used to conduct a quality check for all the DNA samples. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA. Subsequently the DNA concentration was quantified using Qubit 4.0 Fluorometer (ThermoFisher Scientific, Waltham, MA). DNA libraries of each sample were prepared for whole genome sequencing (WGS) using a Nextera XT kit (Illumina, San Diego, CA). Briefly, 0.3 ng/µl of DNA from each *E. coli* isolate was pooled together and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA) using 2 x 250 or 2 x 300 paired-end approach. The raw paired end reads from the sequencer were demultiplexed and submitted to the NCBI database where they were assigned an accession number [21].

Assembly and assessment of genes

E. coli genome sequences were assembled *de novo* using SPAdes version 3.15.4 via a web-based genome assembly service provided by the Bacterial and Viral Bioinformatics Resource Center accessed online at https://www.bv-brc.org/app/Assembly2. The *in silico* analysis of acquired resistance genes and replicon typing for each *E. coli* isolate was used to identify AMR genes harbored on plasmid replicons and was conducted using the Mobile Element Finder tool (database version 1.0.2, 2020-06-09) accessed online via the Center for Genomic Epidemiology (CGE) website (https://cge.food.dtu.dk/services/MobileElementFinder/) [22]. From the output generated, if an isolate had at least one gene from either of the following sets: (i) *ompT* and

hlyF, and (ii) sitABCD, it was deemed ColV plasmid positive [23]. The Mobile Element Finder tool interfaced with the ResFinder 4.1 tool to match individual genes for each *E. coli* isolate to an annotated resistance gene using a 90–100% identity, 60% minimum length, and 90% threshold [22]. PlasmidFinder 2.1 tool (database version 2023-01-18) set at a 95% minimum identity and 60% coverage was used for replicon typing. The CGE pMLST 2.0 tool (database version 2023-04-24) set at a 95% minimum identity and based on IncF-RST configuration was used for *in silico* plasmid MLST typing to determine the plasmid replicon sequence types [24]. We defined molecular MDR as the presence of resistance genes conferring AMR to three or more distinct classes of antimicrobials in the database.

Multi-locus sequence typing (MLST) of *E. coli* isolates

In silico prediction of MLST was performed by submitting the assembled genome of an isolate to the *E. coli* PubMLST database (https://pubmed.ncbi.nlm.nih.gov/30345391/). The MLST 2.0 (2022-11-14) tool on CGE website analyzed the contigs using previously described schemes by Achtman to assign sequence types (STs) based on allelic variations amongst seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) [25]. *E. coli* isolates with identical sequences at all seven loci were assigned STs however those without perfect matches were usually identified as novel or unknown.

Determination of *E. coli* phylogroups and phylogeny

The phylogenetic classification of the *E. coli* genomes was conducted using *in silico* Clermon-Typing 1.4.1 tool as previously described [26]. The ClermonTyper web interface is freely accessible at http://clermontyping.iame-research. center/. The Nextflow workflow was used to map the *E. coli* whole genome fastq pair end reads to a reference genome as well as call variants which were used to generate a maximum likelihood phylogenetic tree (https://gitlab.com/cgps/ghru/pipelines/snp_phylogeny). The clonal relationship between isolates was estimated using a pairwise single nucleotide polymorphism (SNP) analysis. The SNP analysis was used to determine how closely related the isolates were using 'snp-dists', a command line bioinformatics tool for transforming multiple DNA sequence alignment into a distance matrix (https://github.com/tseemann/snp-dists). Isolates that were less than 30 SNPs apart were related. The maximum likelihood phylogenetic tree was visualized using the interactive Tree of Life tool–iTOL version 6 (http://itol.embl.de/itol.cgi).

Data analyses

Data were analyzed using R version 4.3 statistical software. The data were summarized by calculating frequencies and proportions. Pearson's chi-squared test and Fisher's exact test were used to compare MDR prevalence across retail meat types and significance was determined at a p-value $\leq$ 0.05. The additional data file for this study contains a list of accession numbers for individual Sequence Read Archive (SRA) for the *E. coli* isolates (S1 File).

Results

From January through December 2022, a total of 312 meat samples were purchased from 48 grocery stores in North Carolina, representing 8 counties. Of these samples, 69 (22.1%) were labeled with "no-antibiotic-ever", 39 (12.5%) were labeled as organic, and 204 (65.3%) were identified as having no antibiotic use claim present. Of the 312 samples, 138 were positive for *E. coli* with an overall prevalence of 44.2%. Of these, 78 (56.5%) *E. coli* isolates were recovered from ground turkey, followed by chicken breast (n = 27, 19.6%), chicken giblets (n = 14,
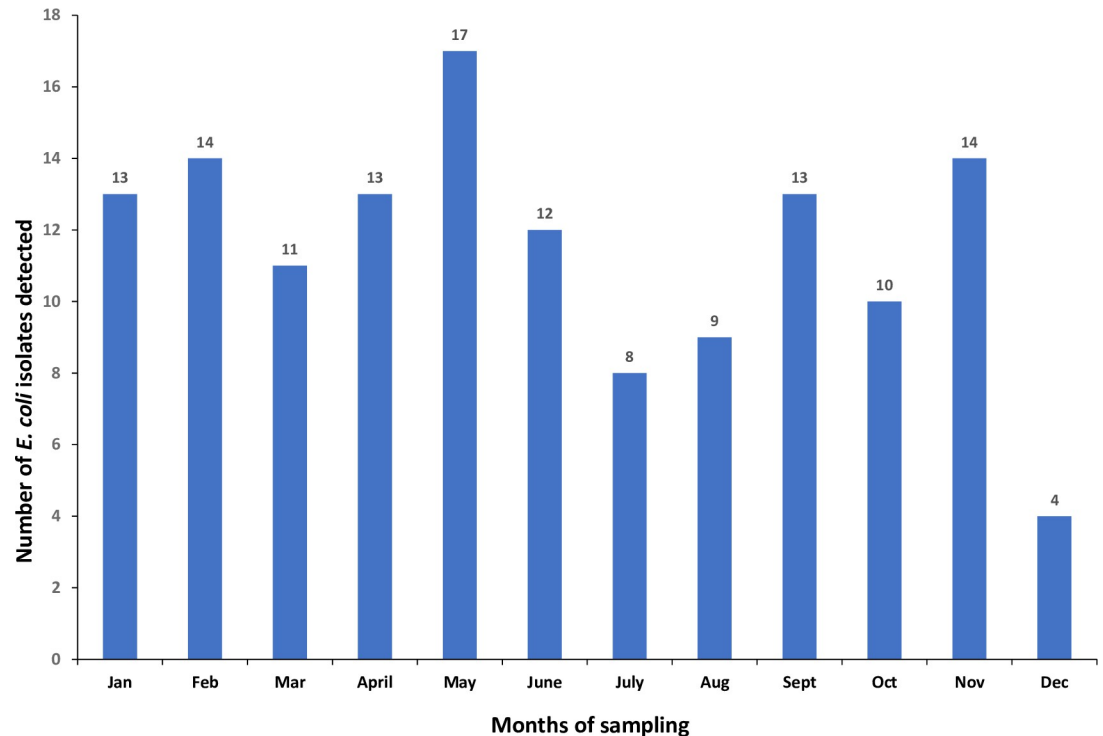
Fig 1. *Escherichia coli* **occurrence in retail meat by month of sampling in North Carolina.** The highest *E. coli* occurrence was observed in May and the lowest in December 2022.

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10.1%) and pork chops (n = 19, 13.8%). The differences observed in prevalence of *E. coli* among retail meat types were statistically significant (X^2 = 13.17; p<0.01). Regardless of meat type, the distribution of *E. coli* isolates across meat types was 15.2% (n = 21) in organic products, 18.8% (n = 26) in "no-antibiotic-ever" products and 65.9% (n = 91) in products with "no claims". The observed prevalence difference was not statistically significant across label claims (X^2 = 2.67; p = 0.26). The prevalence of *E. coli* isolates differed by month of sampling with the highest prevalence observed in May (17/26; 65.3%), followed by February and November (14/26; 53.8% each) while the least prevalence was observed in December (4/26; 15.3%) as shown in Fig 1. This observed difference between months was statistically significant (X^2 = 19.29; p = 0.05). However, there was no significant association between *E. coli* prevalence and season of retail meat purchase from 138 positive isolates (X^2 = 4.73; p = 0.19). The highest prevalence (41/78; 52.6%) was observed in Spring and the lowest prevalence (29/78; 37.2%) was observed in Summer.

Of all *E. coli* isolates, 84.8% (117/138) had good quality sequence data available for further characterization. The accession numbers for 117 paired end reads for the NARMS *E. coli* isolates collected in North Carolina by the North Carolina State University (NCSU) College of Veterinary Medicine (CVM) Thakur Molecular Epidemiology Laboratory for 2022 have been uploaded onto the National Center for Biotechnology Information (NCBI) database https://www.ncbi.nlm.nih.gov under bio projects accession number PRJNA292663. Of these, 72 (61.5%) were recovered from ground turkey, 27 (23.1%) from chicken breast and 18 (15.4%) from pork chops. AMR genes were detected in 77.8% (91/117) of isolates, and 35.9% (42/117) were defined as MDR. The distribution of MDR *E. coli* isolates across retail meat type was 83.3% (35/42) for ground turkey, 9.5% (4/42) from chicken breast and 7.1% (3/42) from pork

Table 1. Antimicrobial resistance (AMR) genes detected in *Escherichia coli* isolated from retail meat in North Carolina, USA.

AMR genes	AMR class	Overall n = 117 (%)	Ground turkey n = 72 (%)	Chicken breast n = 27 (%)	Pork chop n = 18 (%)
aac(3)-lld	Aminoglycosides	3 (2.6)	3 (4.2)	0 (0)	0 (0)
aac(3)-IV		15 (12.8)	14 (19.4)	1 (3.7)	0 (0)
aac(3)-Vla		5 (4.3)	3 (4.2)	2 (7.4)	0 (0)
aadA1		15 (12.8)	9 (12.5)	3 (11.1)	3 (16.7)
aph(3")-lb		29 (24.8)	24 (33.3)	2 (7.4)	3 (16.7)
aph(4)-la		15 (12.8)	15 (20.8)	0 (0)	0 (0)
aph(6)-ld		25 (21.4)	20 (27.8)	2 (7.4)	3 (16.7)
*bla*CARB-2	Beta-lactamases	1 (0.9)	1 (1.4)	0 (0)	0 (0)
*bla*HERA-3		9 (7.7)	9 (12.5)	0 (0)	0 (0)
*bla*OXA-1		1 (0.9)	1 (1.4)	0 (0)	0 (0)
*bla*TEM-1		24 (20.5)	20 (27.8)	2 (7.4)	2 (11.1)
*bla*TEM-141		1 (0.9)	1 (1.4)	0 (0)	0 (0)
*bla*TEM-206		1 (0.9)	1 (1.4)	0 (0)	0 (0)
dfrA1	Folate pathway antagonists	2 (1.7)	1 (1.4)	1 (3.7)	0 (0)
dfrA14		1 (0.9)	1 (1.4)	0 (0)	0 (0)
dfrA15		1 (0.9)	0 (0)	0 (0)	1 (5.6)
sul1		11 (9.4)	8 (11.1)	2 (7.4)	1 (5.6)
sul2		14 (12.0)	11 (15.3)	3 (11.1)	0 (0)
floR	Phenicols	1 (0.9)	1 (1.4)	0 (0)	0 (0)
fosA7	Phosphonic antibiotics	1 (0.9)	1 (1.4)	0 (0)	0 (0)
qacE	Quinolones	8 (6.8)	5 (6.9)	2 (7.4)	1 (5.6)
qacL		2 (1.7)	2 (2.8)	0 (0)	0 (0)
qnrB19		2 (1.7)	2 (2.8)	0 (0)	0 (0)
qnrS1		1 (0.9)	1 (1.4)	0 (0)	0 (0)
Inu(F)	Macrolides	2 (1.7)	0 (0)	0 (0)	2 (11.1)
tetA	Tetracyclines	29 (24.8)	23 (31.9)	5 (18.5)	1 (5.6)
tetB		41 (35.0)	36 (50.0)	2 (7.4)	3 (16.7)
tet gene (A&B)		60 (51.3)	49 (68.1)	7 (25.9)	4 (22.2)

chop, respectively. Sequencing analysis of AMR genes showed that the most prevalent resistance genes detected in the *E. coli* isolates from retail meats belonged to aminoglycosides (91.5%; 107/117), tetracyclines (59.8%; 70/117), beta-lactamases (31.6%; 37/117), folate pathway antagonists (25.2%; 29/117) and quinolones (11.1%; 13/117) as shown in Table 1.

Within each retail meat type, we assessed the impact of label antibiotic use disclosure as displayed on the packaging on MDR. The different label types did not statistically impact the resistance of MDR *E. coli* isolates (p = 0.33). MDR in turkey *E. coli* isolates was significantly different when compared to all other retail meat types regardless of the label claim (p < 0.01) (Table 2).

The MLST analysis showed that the 117 *E. coli* isolates belonged to 81 known sequence types (STs) with the most prevalent being ST117 (8.5%;10/117), ST297 (5.1%; 6/117), ST58 (n = 4; 3.4%), and three isolates each (2.6%) for ST10, ST126, ST602, and ST1079. Numerous other STs were identified among the *E. coli* isolates with one or two isolates represented; ST131 and ST371 were not detected. The most prevalent MLST types detected in the isolates are shown in Fig 2.

The 117 isolates in this study belonged to nine different phylogroups with the most prevalent phylogroup representing B1 (n = 34; 29.1%) followed by phylogroup A (n = 33; 28.2%), phylogroup B2 (n = 16; 13.7%) and phylogroup G (n = 11; 9.4%). Isolates with phylogroup B1

Table 2. Association of multidrug resistance (MDR) *Escherichia coli* in retail meat with antibiotics use label claims.

Variables		MDR n = 42 (%)	Not MDR n = 75 (%)	Pearson's chi-squared	p-value
Antibiotics use claims on package (all meat types)	No claim on package	29 (69.0)	50 (66.7)	2.21	0.33 0.34*
	Organic	4 (9.5)	14 (18.7)		
	No antibiotics use ever	9 (21.4)	11 (14.7)		
Retail Meat types	Ground Turkey	35 (83.3)	37 (49.3)	13.17	≤ **0.01** < **0.01***
	Chicken breast	4 (9.5)	23 (30.7)		
	Pork chop	3 (7.1)	15 (20.0)		
#Ground Turkey (antibiotics use claims)	No claim on package	26 (61.9)	25 (33.3)	4.39	0.11 0.12*
	Organic	2 (4.8)	8 (10.7)		
	No antibiotics use ever	7 (16.7)	4 (5.3)		
##Ground Turkey (antibiotics use claims)	No claim on package	26 (61.9)	25 (33.3)	0.14	0.71 0.61*
	Claim (Organic/ "no antibiotics ever")	9 (21.4)	12 (16)		

*Fisher's Exact Test; #Analysis of different antibiotics use claims among ground turkey meat products; ##The second analysis compares the difference between "no claim" versus "claim" among ground turkey meat products.

were recovered from ground turkey (19/34; 55.9%), chicken breast (8/34; 23.5%) and pork chop (7/34; 20.6%) (Fig 3). Most isolates assigned ST297 (n = 4) and ST58 (n = 2) belonged to phylogroup A, while half of the isolates assigned ST117 (n = 5) belonged to phylogroup B1.

Thirty-four different replicon types were observed among *E. coli* isolates from retail meat. The most prevalent replicon types were IncFIB(AP001918) (n = 73, 62.4%), Col(MG828) (n = 50, 42.4%) and IncFIC(FII) (n = 43, 36.4%) as shown in Table 3. Thirteen replicon types
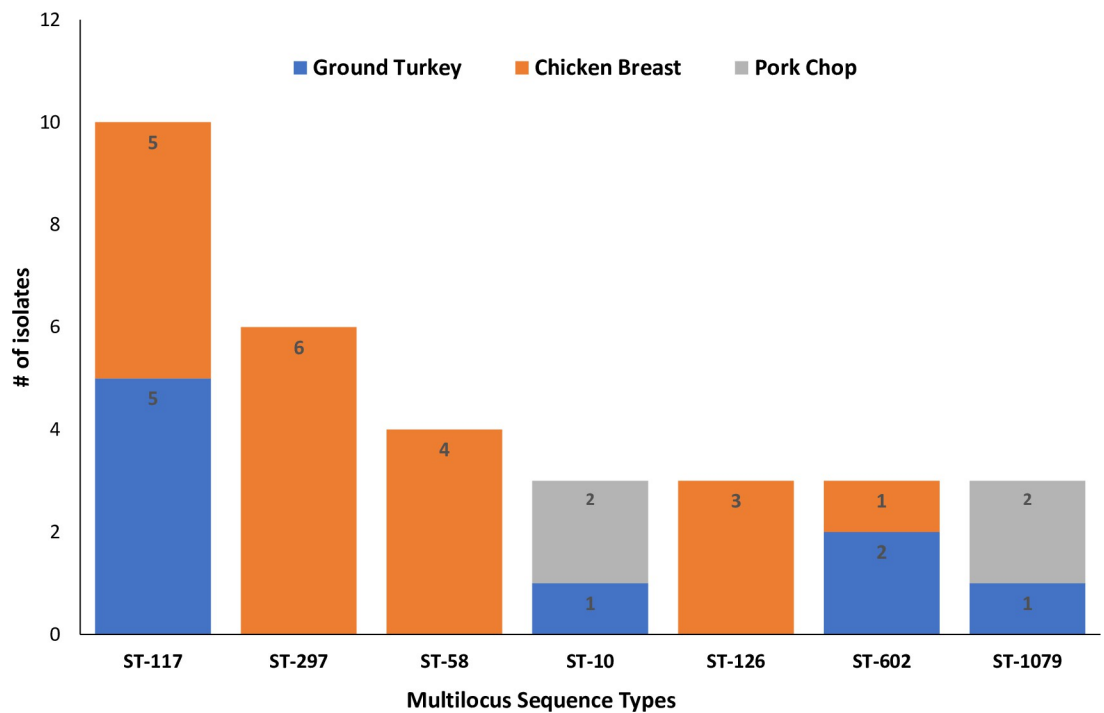

Fig 2. Multi-locus sequence types of *Escherichia coli* isolated from ground turkey, chicken breast, and pork chops in North Carolina, USA. Each bar represents the various *E. coli* sequence types for isolates obtained from the different retail meat sources.
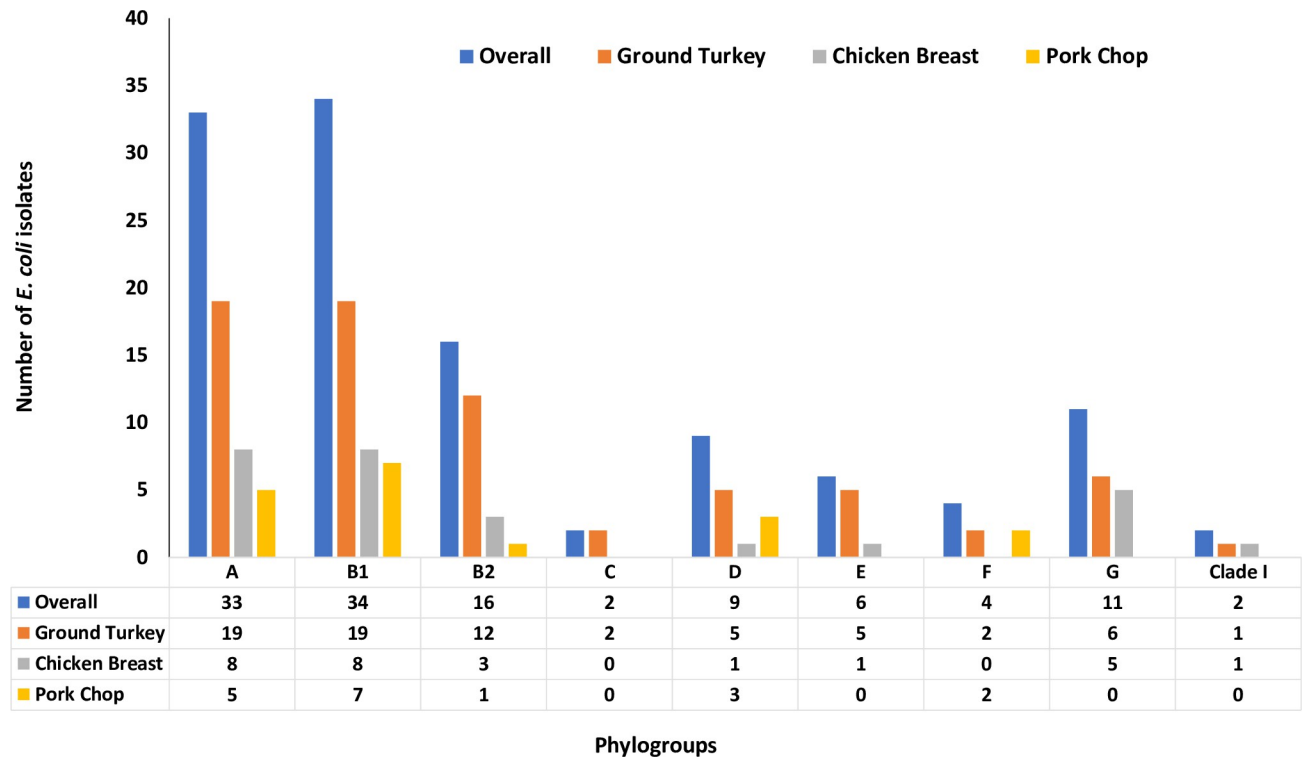
Fig 3. Phylogenetic classification of *Escherichia coli* isolates from retail meat types in North Carolina, 2022.

were common to isolates from all retail meat sources: IncFIB(AP001918), IncFII, IncFIC(FII), IncFII(29), Col(MG828), Col156, ColpVC, Col440I, IncX1, IncFII(pCoo), IncFIA, IncI1, and p0111. IncFIB(AP001918) was the most prevalent in all meat types: ground turkey (n = 51), chicken breast (n = 18) and pork chop (n = 3) followed by Col(MG828) detected in ground turkey (n = 35), chicken breast (n = 12) and pork chop (n = 3).

Of 117 *E. coli* isolates, only 92 (78.6%) had 50 known F plasmid replicon sequence types. The most common were F18:A-:B1 (12/92, 13%); F18:A-:B- (6/92, 6.5%); F4:A-:B20 (6/92, 6.5%); F18:A27:B1 (5/92, 5.4%); F24:A-:B1 (4/92, 4.3%); F18:A-:B20 (3/92, 3.3%); F2:A-:B- (3/92, 3.3%) and F4:A-:B1 (3/92, 3.3%) as shown in S1 Table. The F18:A-:B1 sequence type originated mostly from turkey (8/12) and chicken (4/12) meat products. Eighteen of the isolates did not have any F plasmid replicons while the remaining seven isolates were not typable due to the presence of novel alleles. The prevalent F plasmid replicon sequence types observed in *E. coli* ST117 (n = 10) were F18:A-:B1 (4/10) of which most were from chicken (3/4) and F24:A-:B1 (3/10) from ground turkey (2/3). Plasmid profiling identified presumptive ColV positive plasmid replicons in 72.6% (85/117) of *E. coli* isolates recovered from different retail meat products (S2 Table). Of these, 67.1% (57/85) were recovered from ground turkey, chicken 25.9% (22/85) and pork 7.1% (6/85).

Overall, 117 isolates were used to construct a SNP-based maximum likelihood phylogenetic tree (Fig 4). The *E. coli* isolates from retail meat were very diverse and only isolates from the same meat type were clonally related. More than half (57.3%) of the isolates were clustered into two main phylogroups and seven different STs. The SNPs matrix based on the core genome of 117 *E. coli* strains showed five clonal relationships with a pairwise SNP difference of below 30 (Table 4). *E. coli* isolates with a clonal relationship were clustered together based on phylogroups and STs as shown on the phylogenetic tree in Fig 4.

Table 3. Plasmid replicon types detected in *Escherichia coli* isolates from retail meat types in North Carolina, 2022.

Plasmid replicon types	Overall n = 117 (%)	Ground turkey n = 72 (%)	Chicken breast n = 27 (%)	Pork chop n = 18 (%)
IncFIB(AP001918)	73 (62.4)	51 (70.8)	18 (66.7)	3 (16.7)
Col(MG828)	50 (42.4)	35 (48.6)	12 (44.4)	3 (16.7)
IncFIC(FII)	43 (36.4)	32 (44.4)	10 (37)	1 (5.6)
Col156	37 (31.4)	23 (31.9)	9 (33.3)	5 (27.8)
IncI1	31 (26.3)	25 (34.7)	4 (14.8)	2 (11.1)
p0111	26 (22)	17 (23.6)	7 (25.9)	2 (11.1)
IncFII	25 (21.2)	14 (19.4)	8 (29.6)	3 (16.7)
IncHI2	18 (15.3)	17 (23.6)	1 (3.7)	0 (0)
IncHI2A	18 (15.3)	17 (23.6)	1 (3.7)	0 (0)
Col440I	17 (14.4)	9 (12.5)	3 (11.1)	5 (27.8)
IncFIA	15 (12.7)	9 (12.5)	5 (18.5)	1 (5.6)
IncFIA(HI1)	13 (11)	11 (15.3)	0 (0)	2 (11.1)
IncFII(pSE11)	11(9.3)	8 (11.1)	3 (11.1)	0 (0)
IncFII(pRSB107)	11(9.3)	10 (13.9)	1 (3.7)	0 (0)
ColRNAI	10 (8.5)	6 (8.3)	4 (14.8)	0 (0)
IncX1	9 (7.6)	4 (5.6)	3 (11.1)	2 (11.1)
IncHI1B(R27)	9 (7.6)	9 (12.5)	0 (0)	0 (0)
IncHI1A	9 (7.6)	9 (12.5)	0 (0)	0 (0)
ColpVC	8 (6.8)	2 (2.8)	5 (18.5)	1 (5.6)
IncX4	8 (6.8)	8 (11.1)	0 (0)	0 (0)
IncFII(pCoo)	8 (6.8)	4 (5.6)	2 (7.4)	2 (11.1)
IncY	6 (5.1)	5 (6.9)	1 (3.7)	0 (0)
IncB/O/K/Z	5 (4.2)	0 (0)	0 (0)	5 (27.8)
IncHI1B(CIT)	5 (4.2)	5 (6.9)	0 (0)	0 (0)
IncF(29)	5 (4.2)	2 (2.8)	1 (3.7)	2 (11.1)
IncFIB(K)	5 (4.2)	1 (1.4)	0 (0)	4 (22.2)
IncFII(pHN7A8)	5 (4.2)	3 (4.2)	2 (7.4)	0 (0)
Col8282	4 (3.4)	2 (2.8)	2 (7.4)	0 (0)
IncFIB(pB171)	2 (1.7)	2 (2.8)	0 (0)	0 (0)
IncX2	1 (0.8)	1 (1.4)	0 (0)	1 (5.6)
ColE10	1 (0.8)	0 (0)	0 (0)	1 (5.6)
IncN	1 (0.8)	0 (0)	0 (0)	1 (5.6)
Col(Ye4449)	1 (0.8)	0 (0)	0 (0)	1 (5.6)
IncA/C2	1 (0.8)	1 (1.4)	0 (0)	0 (0)

Seventeen isolates (14.5%) carried AMR genes on plasmid replicons identified on the same assembly scaffold, out of which 88.2% (15/17) were recovered from ground turkey and 11.8% (2/15) from chicken breast. The most prevalent replicon types were IncHI2 and IncHI2A harboring aminoglycoside resistance gene *aph(3")-Ib* in eight isolates (47.1%) and in combination with *aph(6)-Id* in three of the eight isolates recovered from ground turkey. Other AMR genes carried on plasmids include *bla*TEM-1A on IncI-1; *qacE + sul1* on IncFIC (FII); *qnrB19* on Col440I amongst others (Table 5).

Discussion

In this study, we assessed the current prevalence and describe the characteristics of clonal types and AMR in *E. coli* isolates from retail meat, including chicken breast, ground turkey
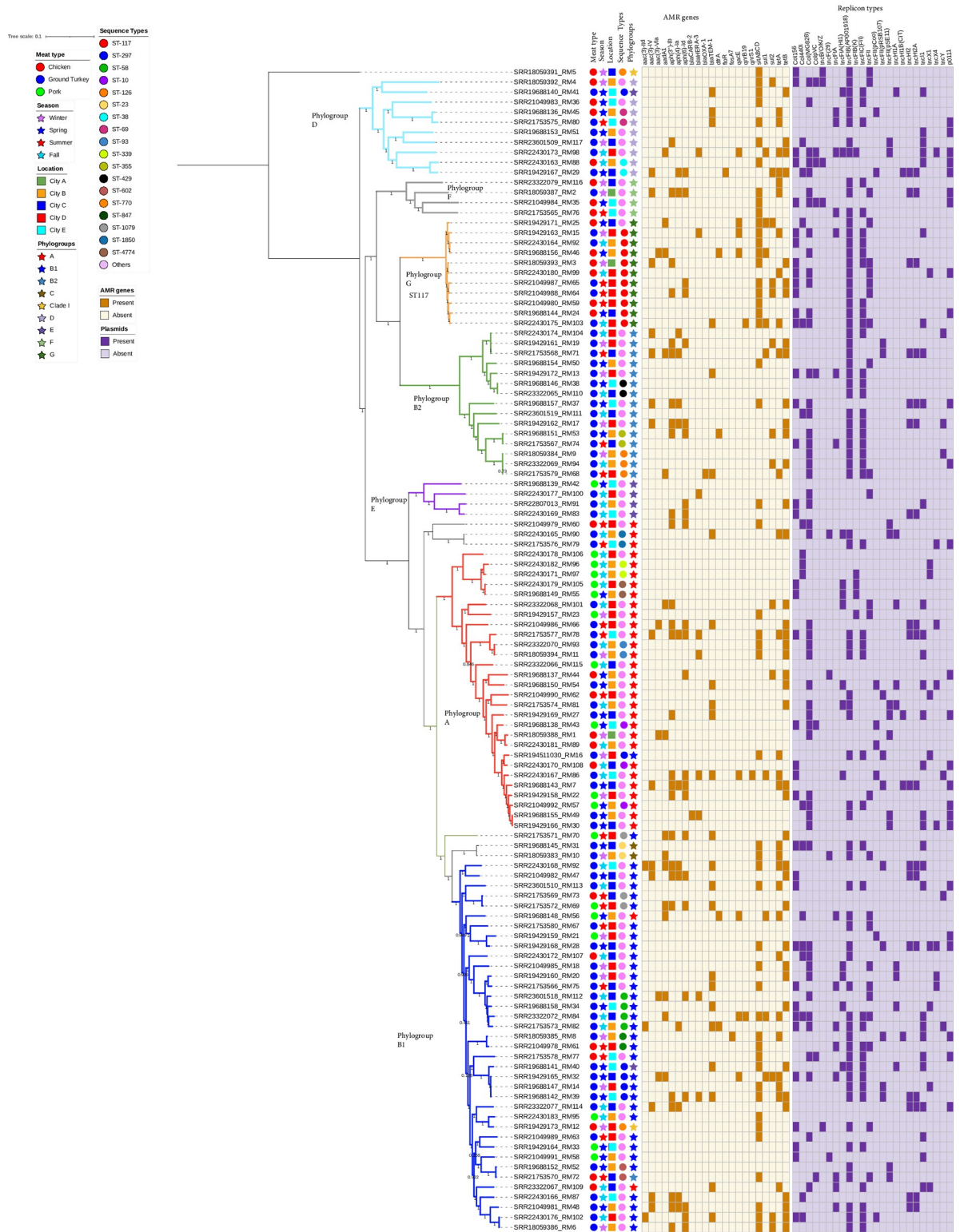
Fig 4. SNP-based phylogeny of *Escherichia coli* isolates from retail meat in North Carolina, USA, 2022. SNP-based maximum likelihood phylogeny of E. coli isolates visualized in interactive Tree of Life tool (iTOL). The tree was rooted in a reference *E. coli* strain K-12 MG1655. Clustering of isolates was found to be following the core genome and SNP-based phylogenies as clustering of isolates with the same sequence types and phylogroups was consistent. Shown for each isolate is the meat type, season, phylogroups, sequence types, AMR genes (brown) and plasmids (purple).

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Table 4. Clonal relationship between *Escherichia coli* isolates from different sources.

Clonal relationship	Sample ID	Retail meat sources	SNP Difference
A	RM68 and RM94	Ground Turkey	13
B	RM9 and RM68	Ground Turkey	14
C	RM9 and RM94	Ground Turkey	17
D	RM105 and RM55	Pork chop	18
E	RM38 and RM110	Ground Turkey	20

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and pork chops purchased from retail grocery stores in North Carolina, USA. Using labeling claims, we were able to assess the role of antibiotic use on the occurrence and characteristics of AMR. We found the presence of AMR genes against different antimicrobial drug classes (aminoglycosides, tetracyclines, beta-lactamases, folate pathway antagonists, quinolones, and macrolides) in *E. coli* isolates from chicken, turkey and pork sources, but the prevalence differed by the various retail meat types. Our study results are consistent with historical genotypic prevalence reported in previous NARMS surveillance program [16–18].

In the present study, the highest prevalence of *E. coli* in the retail meat types was observed in May (spring). Our results show that there was no statistically significant association between the season of retail meat purchase and the detection of *E. coli*, although *E. coli* prevalence did differ by the month of sampling. The role of season on the presence of *E. coli* in retail meats is unclear as reports of another NARMS study in California found a prevalence of 36.7% in spring when compared to winter [17], while a similar study done in Canada found no definite seasonal trend [27]. The interplay between the effect of season and the prevalence of *E. coli* in retail meat is not fully understood [27].

Table 5. Antimicrobial resistance (AMR) genes carried on plasmid replicon detected in 17 *Escherichia coli* isolates from retail meat, North Carolina, USA, 2022.

SRR#	Retail Meat types	Sequence Types	Plasmid replicon	AMR genes harbored on plasmids
SRR19429162	Ground Turkey	ST141	IncHI2, IncHI2A	*aph(3")-Ib + aph(6)-Id*
SRR19429165	Ground Turkey	ST297	IncI-1	*bla*TEM-1A
SRR19688157	Ground Turkey	ST3672	IncHI2, IncHI2A	*aph(3")-Ib + aph(3")-Ib*
SRR19688156	Chicken breast	ST117	IncFIC(FII)	*qacE + sul1*
SRR21049981	Ground Turkey	ST162	IncHI2, IncHI2A	*aph(3")-Ib + aph(6)-Id*
SRR21049979	Chicken breast	ST1771	IncFII(pSE11)	*aph(3")-Ib + aph(6)-Id*
SRR21753579	Ground Turkey	ST126	IncFII	*bla*TEM-1A
SRR21753568	Ground Turkey	ST11991	IncHI2, IncHI2A	*aph(3")-Ib + aph(3")-Ib*
SRR21753577	Ground Turkey	ST2253	IncHI2, IncHI2A	*aph(3")-Ib + aph(6)-Id*
SRR23322072	Ground Turkey	ST58	Col440I	*qnrB19*
SRR22430168	Ground Turkey	ST351	IncHI2, IncHI2A	*aph(3")-Ib + aph(3")-Ib*
SRR22430167*	Ground Turkey	ST13930	IncX2	*aph(3')-Ia*
SRR22430167*	Ground Turkey	ST13930	IncY	*aph(3")-Ib + aph(6)-Id*
SRR22430166	Ground Turkey	ST4038	IncHI2, IncHI2A	*aph(3")-Ib + aph(3")-Ib*
SRR22430173	Ground Turkey	ST1938	IncX4	*bla*TEM-141 + *bla*TEM-206
SRR22430176	Ground Turkey	ST12733	IncFII(pCoo)	*aac(3)-IId + blaTEM-1B*
SRR23601518*	Ground Turkey	ST58	IncFII	*bla*HERA-3
SRR23601518*	Ground Turkey	ST58	IncA/C2	*aadA1 + aac(3')-VIa*
SRR23322077	Ground Turkey	ST14287	IncHI2, IncHI2A	*aph(3")-Ib + aph(3")-Ib*

* *E. coli* isolate with the same SRR#.

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Across all isolates from all retail meat sources, we found that there was a high proportion of genotypic AMR (77.8%) among *E. coli* and a moderate occurrence of MDR *E. coli* (35.9%); this prevalence is consistent with previous data collected from retail meat in the United States over the last decade [9, 17, 19, 28, 29]. The prevalence of resistance was highest in *E. coli* from retail ground turkey when compared to other meat types. This also is consistent with findings from similar studies in Arizona which reported a high resistance prevalence of *E. coli* (90.7%) in retail turkey [19] and California reporting a prevalence of 70.4% [17]. This suggests there are differences in the management and production of turkey which may select for antimicrobial resistance or allow its persistence. It is important to note that producers in the United States are not required to make public reports on antimicrobial use data, hence it is difficult to link on-farm use of antimicrobials to development of AMR. Although between 2013 and 2017, the largest US turkey production corporations significantly reduced their overall use of antimicrobials attributed to the full implementation of FDA guidance for industry (GFI) #213, and improved antimicrobial stewardship amongst other factors [30].

The prevalence of genotypic resistance for ampicillin detected in *E. coli* from all meat types (31.6%; 37/117) was higher than the national average (11.6%; 54/466) for 2022; however, the prevalence of genotypic resistance for tetracycline was not different in *E. coli* from ground turkey (68.1%; 49/72) when compared to the national average of 62.5% (619/990). This was lower for chicken (25.9%; 7/27) and pork (22.2%; 4/18) when compared to the national average of 31.5% (147/466) and 50.4% (192/381) respectively [9]. In addition, to the ampicillin and tetracycline resistance genes, our results also show sulfonamide resistance genes and plasmid mediated quinolone resistance genes detected in retail meat. This finding aligns with past reports of the NARMS surveillance program [16–18].

Our data indicated that retail ground turkey may serve as an important source of MDR *E. coli* with the potential for transmission to humans if proper food handling practices are not used. However, there was no significant association between MDR *E. coli*, and the different antibiotics-use claims on the meat package labels. High resistance rates observed among *E. coli* isolates from turkey with "no antibiotic-use claims" may indicate that they were raised using conventional methods, however, this cannot be confirmed. This is consistent with the reports of a similar study in Arizona, USA [19]. In conventional turkey production, while there may be a greater potential for antimicrobial use when compared to "no antibiotics ever" and "organic" production, we are not able to definitively determine the use.

The *E. coli* isolates from retail meat samples displayed diverse STs and phylogroups suggesting the possibility that the isolates had evolved overtime from different *E. coli* clones. The most prevalent known STs among the isolates were ST117 (recovered from chicken and turkey), ST297 and ST58 (both recovered from chicken breast). ST10 was also detected in isolates from turkey and pork. A study conducted in California, USA in 2018 identified ST117 and ST10 in *E. coli* isolates from retail meat samples and humans [31]. Our results are consistent with reports of another related study that detected ST117 in *E. coli* recovered from commercial chicken and turkey [32] but at variance with others [32, 33] that reported ST58 and ST10 were dominant in turkey clinical *E. coli* isolates. The most dominant phylogroups in the present study were phylogroups A, B1 and B2 which was detected in isolates from all retail meat types and consistent with the literature [18, 34, 35].

The different replicon types along with the numerous *E. coli* STs in the present study highlight the diversity and complex nature of these indicator organisms. The most prevalent replicon types observed were IncFIB(AP001918), Col(MG828), IncFIC(FII), Col156, IncI1, p0111, IncFII, IncHI2, IncHI2A, Col440I and IncFIA. Seventeen isolates carried AMR genes on plasmid replicon identified on the same assembly scaffold. Replicon typing showed IncHI2 and IncHI2A harbored AMR gene *aph(3")-Ib* + *aph(6)-Id* in some isolates recovered from ground

turkey. Interestingly, the IncFIC(FII) plasmid was observed to harbor the *qacE* and *sul1* AMR gene on the assembly scaffold originating from one *E. coli* ST117 strain, recovered from retail chicken meat in the present study. This is of concern as ST117 is a known pathogenic *E. coli* lineage that has been implicated in both human and animal diseases [31, 36]. Other AMR genes carried on plasmid contigs in our study include *bla*TEM-1A on IncI-1; *qacE* + *sul1* on IncFIC (FII); *qnrB19* on Col440I amongst others. Other studies have reported *E. coli* isolates from food animals carrying the plasmid mediated quinolone resistance gene *qnrB* on a Col440I replicon carrying plasmid contig supporting our study results [37, 38].

Our analysis showed that F18:A-:B1 was the most commonly detected plasmid replicon sequence type and originated mostly from ground turkey meat products. Although these sequence types are associated with pathogenic *E. coli* causing diseases in poultry, they have been detected in faecal *E. coli* originating from healthy poultry [39, 40]. Furthermore, this study shows that ColV plasmids were harbored in *E. coli* isolated from retail meat especially ground turkey and chicken. This is not surprising because other studies have reported that ceacal *E. coli* recovered from healthy poultry were observed to carry ColV plasmids [39, 40]. In addition, avian-associated ColV plasmids have been recovered from *E. coli* originating from poultry meat products further supporting our claims [23].

Studies have reported a high diversity between *E. coli* isolates recovered from retail meat types as evident by the wide variety of STs observed [31, 41]. The *E. coli* isolated from retail meat in the present study were genetically diverse hence we did not detect any clonal relationship between isolates from the different meat types. However, our results show that isolates from the same meat type i.e., ground turkey were found to be closely related within 20 SNPs.

This study is not without limitations, particularly the small number of *E. coli* isolates characterized by sequencing may not fully encompass the diversity of *E. coli* strains recovered from retail meats, although it is a representative random sampling. Another limitation of retail level surveillance used in this study is the inconsistent or limited availability of metadata for each sample. This impacts the ability to assess factors along the food production chain from farm-to-fork that can potentially contribute to AMR and MDR in *E. coli* recovered from retail meat products. Nevertheless, meat type, label claim, packaging and season only accounted for a small portion of the variability in AMR genetic determinants among the *E. coli* isolates in this investigation, which were genotypically heterogeneous with regards to AMR.

Conclusions

In conclusion, we detected MDR *E. coli* from retail meat types in North Carolina. Our results showed that > 40% of the retail meats purchased from grocery stores were contaminated with *E. coli*, and of these the vast majority were resistant to aminoglycosides (*aph(3")-Ib & aph(6)-Id)*, tetracycline (*tetA & tetB*) or ampicillin (*bla*TEM-1). The resistance prevalence was highest among *E. coli* isolates from turkey for most AMR genes detected. Our data indicated that ground turkey may serve as an important source of MDR *E. coli*. The isolates were diverse as only ten showed clonal relationships with a pairwise difference of ≤ 20 SNPs.

Ten of the *E. coli* isolated from retail meat had ST117 which is an emerging sequence type implicated as a human pathogen. It is important to emphasize that the bacteria isolated from retail meat samples in the present study were generic *E. coli* hence the risk of impacting negatively on human health is quite low. However, we cannot rule out the possibility of horizontal gene transfer between *E. coli* strains because our results show that plasmids, which are mobile genetic elements harbored some AMR genes which should be a source of concern for human health. Therefore, surveillance of these indicator bacteria strains should continue to serve as a warning for preventing the spread of AMR along the food chain. To further understand the

transmission dynamics, additional studies are required especially due to the persistence of these acquired AMR genes and plasmid replicon types in different *E. coli* STs.

Supporting information

S1 File. This file contains the metadata, resistance genes and plasmids data for the retail meat isolates. The metadata comprises the NCBI sequence SRR, sampling month, location, meat type, meat cut, raising claim, process date, packaging type and collection year.
(XLSX)

S1 Table. Distribution of F plasmid replicon sequence types in *E. coli* isolated from retail meat products, North Carolina.
(DOCX)

S2 Table. Plasmid profiling of ColV plasmid replicons in *E. coli* isolates recovered from retail meat products, North Carolina.
(XLSX)

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Author Contributions

References

1. Tacconelli E, Sifakis F, Harbarth S, Schrijver R, van Mourik M, Voss A, et al. Surveillance for control of antimicrobial resistance. Lancet Infect Dis. 2018; 18:e99–106. https://doi.org/10.1016/S1473-3099(17)30485-1 PMID: 29102325

2. McEwen SA, Collignon PJ. Antimicrobial resistance: A One Health perspective. Microbiol Spectr. 2018; 6:1–26. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017 PMID: 29600770

3. Manges AR. Escherichia coli and urinary tract infections: The role of poultry-meat. Clin Microbiol Infect. 2016; 22:122–9. https://doi.org/10.1016/j.cmi.2015.11.010 PMID: 26679924

4. Buberg ML, Mo SS, Sekse C, Sunde M, Wasteson Y, Witsø IL. Population structure and uropathogenic potential of extended-spectrum cephalosporin-resistant Escherichia coli from retail chicken meat. BMC Microbiol. 2021; 21:1–15. https://doi.org/10.1186/s12866-021-02160-y.

5. Painter JA, Hoekstra RM, Ayers T, Tauxe R V., Braden CR, Angulo FJ, et al. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis. 2013; 19:407–15. https://doi.org/10.3201/eid1903.111866 PMID: 23622497

6. Kim K, Kwon S-K, Kim P, Kim JF. Transcriptional Potential Determines the Adaptability of Escherichia coli Strains with Different Fitness Backgrounds. Microbiol Spectr. 2022; 10:6. https://doi.org/10.1128/spectrum.02528-22 PMID: 36445144

7. Mogren L, Windstam S, Boqvist S, Vågsholm I, Söderqvist K, Rosberg AK, et al. The hurdle approach-A holistic concept for controlling food safety risks associated with pathogenic bacterial contamination of leafy green vegetables. A review. Front Microbiol. 2018;9 AUG:1–20. https://doi.org/10.3389/fmicb.2018.01965

8. Karp BE, Tate H, Plumblee JR, Dessai U, Whichard JM, Thacker EL, et al. National antimicrobial resistance monitoring system: Two decades of advancing public health through integrated surveillance of antimicrobial resistance. Foodborne Pathog Dis. 2017; 14:545–57. https://doi.org/10.1089/fpd.2017.2283 PMID: 28792800

9. FDA. Food and Drug Administration. NARMS Now: Integrated Data. 2023;:1–3. https://www.fda.gov/animal-veterinary/ national-antimicrobial-resistance-monitoring-system/narms-now-integrated-data. Accessed 22 Sep 2023.

10. Bowman M, Marshall KK, Kuchler F, Lynch L. Raised Without Antibiotics: Lessons from Voluntary Labeling of Antibiotic Use Practices in the Broiler Industry. Am J Agric Econ. 2016; 98:622–42. https://doi.org/10.1093/ajae/aaw008

11. Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, Alpuche-Aranda CM. Antimicrobial resistance: One Health approach. Vet World. 2022; 15:743–9. https://doi.org/10.14202/vetworld.2022.743-749 PMID: 35497962

12. Van TTH, Yidana Z, Smooker PM, Coloe PJ. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. J Glob Antimicrob Resist. 2020; 20:170–7. https://doi.org/10.1016/j.jgar.2019.07.031 PMID: 31401170

13. Wallinga D, Smit LAM, Davis MF, Casey JA, Nachman KE. A Review of the Effectiveness of Current US Policies on Antimicrobial Use in Meat and Poultry Production. Curr Environ Heal Reports. 2022;:339–54. https://doi.org/10.1007/s40572-022-00351-x PMID: 35477845

14. Wee BA, Muloi DM, van Bunnik BAD. Quantifying the transmission of antimicrobial resistance at the human and livestock interface with genomics. Clin Microbiol Infect. 2020; 26:1612–6. https://doi.org/10.1016/j.cmi.2020.09.019 PMID: 32979568

15. Kim S, Kim H, Kim Y, Kim M, Kwak H, Ryu S. Whole-genome sequencing-based characteristics in extended-spectrum beta-lactamase-producing escherichia coli isolated from retail meats in Korea. Microorganisms. 2020; 8:1–15. https://doi.org/10.3390/microorganisms8040508 PMID: 32252466

16. NARMS. 2019 NARMS Update: Integrated Report Summary. 2019.

17. Lee KY, Lavelle K, Huang A, Atwill ER, Pitesky M, Li X. Assessment of Prevalence and Diversity of Antimicrobial Resistant Escherichia coli from Retail Meats in Southern California. Antibiotics. 2023;12. https://doi.org/10.3390/antibiotics12040782 PMID: 37107144

18. Woyda R, Oladeinde A, Abdo Z. Chicken Production and Human Clinical Escherichia coli Isolates Differ in Their Carriage of Antimicrobial Resistance and Virulence Factors. Appl Environ Microbiol. 2023; 89:2. https://doi.org/10.1128/aem.01167-22 PMID: 36651726

19. Davis GS, Waits K, Nordstrom L, Grande H, Weaver B, Papp K, et al. Antibiotic-resistant Escherichia coli from retail poultry meat with different antibiotic use claims. BMC Microbiol. 2018; 18:1:174. https://doi.org/10.1186/s12866-018-1322-5

20. NARMS-FDA. Resources | FDA https://www.fda.gov/animal-veterinary/national-antimicrobial-resistanc. . . NARMS Supplemental Material. 2023;:1–5.

21. Timme R, Shrestha Y, Pfefer T, Morin P, Balkey M, Strain E. Quality control assessment for microbial genomes: GalaxyTrakr MicroRunQC workflow. protocols.io. 2023;:1–18. https://doi.org/10.17504/protocols.io.5jyl8mj16g2w/v5

22. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, Petersen TN. Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder. J Antimicrob Chemother. 2021; 76:101–9. https://doi.org/10.1093/jac/dkaa390 PMID: 33009809

23. Liu CM, Stegger M, Aziz M, Johnson TJ, Waits K, Nordstrom L, et al. Escherichia coli ST131-H22 as a Foodborne Uropathogen. MBio. 2018; 9:1–11. https://doi.org/10.1128/mBio.00470-18 PMID: 30154256

24. Clausen PTLC Aarestrup FM, Rapid Lund O. and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics. 2018; 19:307. https://doi.org/10.1186/s12859-018-2336-6

25. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in Escherichia coli: An evolutionary perspective. Mol Microbiol. 2006; 60:1136–51. https://doi.org/10.1111/j.1365-2958.2006.05172.x PMID: 16689791

26. Beghain J, Bridier-Nahmias A, Nagard H Le, Denamur E, Clermont O. ClermonTyping: An easy-to-use and accurate in silico method for Escherichia genus strain phylotyping. Microb Genomics. 2018;4. https://doi.org/10.1099/mgen.0.000192 PMID: 29916797

27. Smith BA, Meadows S, Meyers R, Parmley EJ, Fazil A. Seasonality and zoonotic foodborne pathogens in Canada: Relationships between climate and Campylobacter, E. coli and Salmonella in meat products. Epidemiol Infect. 2019;147. https://doi.org/10.1017/S0950268819000797 PMID: 31364535

28. Tyson GH, Li C, Hsu C-H, Bodeis-Jones S, McDermott PF. Diverse Fluoroquinolone Resistance Plasmids From Retail Meat E. coli in the United States. Front Microbiol. 2019; 10:2826. https://doi.org/10.3389/fmicb.2019.02826 PMID: 31866986

29. Zhao S, Blickenstaff K, Bodeis-Jones S, Gaines SA, Tong E, McDermott PF. Comparison of the prevalences and antimicrobial resistances of Escherichia coli isolates from different retail meats in the United States, 2002 to 2008. Appl Environ Microbiol. 2012; 78:1701–7. https://doi.org/10.1128/AEM.07522-11 PMID: 22247155

30. Singer RS, Porter LJ, Apley MD, Bjork K, Schrag NFD, Davies PR. Estimates of on-farm antimicrobial usage in turkey production in the United States, 2013–2017. Zoonoses Public Health. 2020;67 Nov:36–50. https://doi.org/10.1111/zph.12763 PMID: 33201604

31. Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, McDermott P, et al. A Population-Based Surveillance Study of Shared Genotypes of Escherichia coli Isolates from Retail Meat and Suspected Cases of Urinary Tract Infections. mSphere. 2018;3. https://doi.org/10.1128/mSphere.00179-18 PMID: 30111626

32. Delago J, Miller EA, Flores-Figueroa C, Munoz-Aguayo J, Cardona C, Smith AH, et al. Survey of clinical and commensal Escherichia coli from commercial broilers and turkeys, with emphasis on high-risk clones using APECTyper. Poult Sci. 2023; 102:102712. https://doi.org/10.1016/j.psj.2023.102712 PMID: 37156077

33. Johnson TJ, Miller EA, Flores-Figueroa C, Munoz-Aguayo J, Cardona C, Fransen K, et al. Refining the definition of the avian pathogenic Escherichia coli (APEC) pathotype through inclusion of high-risk clonal groups. Poult Sci. 2022; 101:102009. https://doi.org/10.1016/j.psj.2022.102009 PMID: 35952599

34. Sacher-Pirklbauer A, Klein-Jöbstl D, Sofka D, Blanc-Potard AB, Hilbert F. Phylogenetic groups and antimicrobial resistance genes in escherichia coli from different meat species. Antibiotics. 2021; 10. https://doi.org/10.3390/antibiotics10121543 PMID: 34943755

35. Xia X, Meng J, Zhao S, Bodeis-Jones S, Gaines SA, Ayers SL, et al. Identification and antimicrobial resistance of extraintestinal pathogenic Escherichia coli from retail meats. J Food Prot. 2011; 74:38–44. https://doi.org/10.4315/0362-028X.JFP-10-251 PMID: 21219761

36. Zhao W, Li W, Du XD, Yao H. Hybrid IncFIA/FIB/FIC(FII) plasmid co-carrying blaNDM-5 and fosA3 from an Escherichia coli ST117 strain of retail chicken. Int J Food Microbiol. 2022; 2:382 December:109914. https://doi.org/10.1016/j.ijfoodmicro.2022.109914 PMID: 36088664

37. Juraschek K, Malekzadah J, Malorny B, Käsbohrer A, Schwarz S, Meemken D, et al. Characterization of qnrB-carrying plasmids from ESBL- and non-ESBL-producing Escherichia coli. BMC Genomics. 2022; 23:1–16. https://doi.org/10.1186/s12864-022-08564-y PMID: 35549890

38. Aworh MK, Kwaga JKP, Hendriksen RS, Okolocha EC, Harrell E, Thakur S. Quinolone-resistant Escherichia coli at the interface between humans, poultry and their shared environment- a potential public health risk. One Heal Outlook. 2023; 5:2. https://doi.org/10.1186/s42522-023-00079-0 PMID: 36855171

39. Mageiros L, Méric G, Bayliss SC, Pensar J, Pascoe B, Mourkas E, et al. Genome evolution and the emergence of pathogenicity in avian Escherichia coli. Nat Commun. 2021; 12:765. https://doi.org/10.1038/s41467-021-20988-w PMID: 33536414

40. Cummins ML, Li D, Ahmad A, Bushell R, Noormohammadi AH, Wijesurendra DS, et al. Whole Genome Sequencing of Avian Pathogenic Escherichia coli Causing Bacterial Chondronecrosis and Osteomyelitis in Australian Poultry. Microorganisms. 2023; 11:6. https://doi.org/10.3390/microorganisms11061513 PMID: 37375015; PMCID: PMC10302171.

41. Castellanos LR, Donado-Godoy P, León M, Clavijo V, Arevalo A, Bernal JF, et al. High heterogeneity of Escherichia coli sequence types harbouring ESBL/AmpC genes on IncI1 plasmids in the Colombian poultry chain. PLoS One. 2017; 12:1–15. https://doi.org/10.1371/journal.pone.0170777 PMID: 28125687; PMCID: PMC5268450.