

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

3 Mabel Kamweli Aworh<sup>1</sup>, Siddhartha Thakur<sup>1</sup>, Catherine Gensler<sup>2</sup>, Erin Harrell<sup>1</sup>, Lyndy Harden<sup>1</sup>,  
4 Paula J. Fedorka- Cray<sup>1</sup>, Megan Jacob<sup>1\*</sup>

5 <sup>1</sup>Department of Population Health and Pathobiology, College of Veterinary Medicine, North  
6 Carolina State University, Raleigh, North Carolina, United States of America

7  
8 <sup>2</sup>Department of Agricultural and Human Sciences, College of Agriculture and Life Sciences,  
9 North Carolina State University, Raleigh, North Carolina, United States of America

10

11 **\*Corresponding author:**

12 Megan Jacob, MS, PhD  
13 Professor, Veterinary Microbiology  
14 Department of Population Health and Pathobiology, North Carolina State University  
15 Email: mejacob@ncsu.edu  
16 Phone: 919.513.6236

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18 **Running Head:** *E. coli* characteristics in NARMS retail meat surveillance

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20 **Keywords:** NARMS, Retail Meat, *E. coli*, AMR, North Carolina

21

## 22 **Abstract**

## 23 **Background**

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

## 28 **Methods**

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

## 37 **Results**

38 Of 312 retail meat samples, 138 (44.2%) were positive for *E. coli*, with turkey (78/138; 56.5%)  
39 demonstrating the highest prevalence. Prevalence was lower in chicken (41/138; 29.7%) and  
40 pork (19/138; 13.8%). Quality sequence data was available from 84.8% (117/138) of the *E. coli*  
41 isolates, which included 72 (61.5%) from turkey, 27 (23.1%) from chicken breast, and 18  
42 (15.4%) from pork. Genes associated with AMR were detected in 77.8% (91/117) of the isolates  
43 and 35.9% (42/117) were defined as MDR ( $\geq 3$  distinct classes of antimicrobials). Commonly

44 observed AMR genes included *tetB* (35%), *tetA* (24.8%), *aph(3'')-Ib* (24.8%), and *bla*TEM-1  
45 (20.5%), the majority of which originated from turkey isolates. Antibiotics use claims had no  
46 statistical effect on MDR *E. coli* isolates from the different meat types ( $\chi^2=2.21$ ,  $p=0.33$ ). MDR  
47 was observed in isolates from meat products with labels indicating “no claims” (n=29; 69%),  
48 “no antibiotics ever” (n=9; 21.4%), and “organic” (n=4; 9.5%). Thirty-four different replicon types  
49 were observed. AMR genes were carried on plasmids in 17 *E. coli* isolates, of which 15 (88.2%)  
50 were from turkey and two (11.8%) from chicken. Known sequence types (STs) were described  
51 for 81 *E. coli* isolates, with ST117 (8.5%), ST297 (5.1%), and ST58 (3.4%) being the most  
52 prevalent across retail meat types. The most prevalent phylogroups were B1 (29.1%) and A  
53 (28.2%). Five clonal patterns were detected among isolates.

## 54 **Conclusions**

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

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## 67 **Introduction**

68 Surveillance of antimicrobial resistance (AMR) along the one-health continuum, including in the  
69 food chain, is necessary to reduce the impact of resistant bacteria on health and prioritize  
70 policies and areas of intervention. The value and importance of one-health surveillance  
71 systems have been well described [1]. *Escherichia coli*, a gram-negative bacteria common to  
72 the human and animal gastrointestinal tract, is often used as an indicator organism in AMR  
73 surveillance systems [2]. Studies have shown that food products are potential reservoirs of  
74 pathogenic *E. coli* for humans [3, 4]. Most foodborne outbreaks in humans caused by *E. coli*  
75 have been associated with the consumption of contaminated food products of animal origin or  
76 contaminated with animal feces [5]; additionally, the role of these organisms in transmission of  
77 AMR to naturally-occurring human strains remains unknown and a concern. *E. coli* have flexible  
78 fitness mechanisms, making them easily adaptable to environmental conditions [6]. They can  
79 persist on surfaces for long periods of time and can be isolated from ready-to-eat food products  
80 [7].

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82 To track AMR in food products of animal origin in the United States, the National Antimicrobial  
83 Resistance Monitoring System (NARMS) was established in 1996. The NARMS program which  
84 is operated by the Centers for Disease Control and Prevention (CDC), Food and Drug  
85 Administration (FDA) and U.S. Department of Agriculture (USDA) routinely conducts  
86 surveillance of foodborne pathogens in humans, retail meat products and food animals  
87 respectively. *E. coli* is one of the indicator bacteria identified for AMR surveillance in NARMS  
88 [8]. The NARMS program contributes to the promotion and protection of public health by  
89 disseminating knowledge regarding new bacterial resistance, the distinction between resistant  
90 and susceptible illnesses, and the effects of treatments meant to stop the spread of resistance

91 [9]. Since its inception, the NARMS program has been solely responsible for continued tracking  
92 of antimicrobial resistance among human and animal related populations through the food  
93 supply.

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95 The use of antimicrobials in any setting has potential implications on the development and  
96 maintenance of AMR. In the United States, retail food products including meat have been  
97 marketed with antibiotic use claims since the mid-2000s [10]. There are public concerns that  
98 the use of antimicrobials in food producing animals may affect the efficacy of similar drugs in  
99 human medicine especially through selection pressure of resistant bacteria and their ability to  
100 be transferred to humans through the food chain [11, 12]. The scientific evidence to support  
101 the risk of food products in disseminating AMR bacteria, including *E. coli*, is less clear. Studies  
102 have shown that resistance genes in bacteria such as *E. coli* can be transmitted at the human-  
103 animal-environment interface [13, 14]. Increasingly, modern molecular methods such as whole-  
104 genome sequencing (WGS) have been used to detect and trace the presence of resistance  
105 genes, mobile genetic elements and plasmids in *E. coli*, including from retail meat [15]. The  
106 NARMS surveillance program uses a WGS-based method for evaluating AMR in *E. coli* isolates  
107 from retail meat products.

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109 Historically, the most prevalent AMR genes detected in *E. coli* from retail meat from the NARMS  
110 surveillance program include genes associated with erythromycin (*mph(A)*), tetracycline (*tet(A)*,  
111 *tet(B)* and *tet(C)*), sulfonamide (*sul1* and *sul2*) and plasmid mediated quinolone (*qnr*; *gyr* or *par*  
112 mutations) resistance [16–18]. Recent analysis of the NARMS Genome Trackr database  
113 showed that common phylogroups detected in *E. coli* recovered from retail meat were A, B1,  
114 B2, C, Clade I, D, E, F, and G [18]. Previous NARMS studies have reported *E. coli* prevalence  
115 of 47.5% in all retail meat products, with higher prevalence (90.7%) reported in turkey products

116 [17, 19]. While one study reports more than 50% of the isolates demonstrated multidrug  
117 resistance (MDR), which showed turkey meat-derived *E. coli* isolates with the highest  
118 resistance other studies have reported a lower MDR prevalence of 14.3% [17, 19].

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

## 123 **Materials and methods**

### 124 **Retail meat sampling**

125 Between January and December 2022, 312 meat products comprised of retail chicken cuts  
126 (breast; bone-in/skin-on; n = 96), ground turkey (n=96), pork chop (n=96) and chicken giblets  
127 (liver, gizzard, or heart; n=24) were purchased from grocery supermarkets across five  
128 municipalities and eight counties in North Carolina in accordance with NARMS project protocol  
129 [20]. Monthly, 26 samples were randomly selected and purchased from grocery stores using  
130 zip codes that geographically represented each location based on US FDA assignment. At  
131 each sampling, eight chicken breast, eight pork chop, eight ground turkey and one package  
132 each of chicken heart, liver or gizzards were purchased.

133 As per the NARMS protocol for 2022, the demographic information collected for each  
134 purchased meat sample included antibiotic use claims apparent on product label, store name,  
135 store location, brand name, sell-by-date, purchase date, lab processing date and season of  
136 retail meat purchase (winter, spring, summer, and fall). Winter is considered December,  
137 January, and February; spring is March through May; summer is June through August; and fall  
138 is September through November. The samples were kept on ice in an isothermal container,

139 during transportation from the grocery stores to the laboratory. On arrival at the lab, samples  
140 were refrigerated at 4°C and processed for detection of multiple bacteria, including *E. coli*.

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## 142 **Bacterial culture**

143 We followed the provided NARMS 2022 retail meat surveillance laboratory protocol; briefly, this  
144 began with 50g of each sample aseptically cut into a stomacher bag where 250 ml of Buffer  
145 Peptone Water (Thermo Fisher Scientific, Waltham, MA) was added, and the sample was  
146 homogenized on a shaker at 200rpm for 15 mins. After mixing, the sample was placed into a  
147 sterile, plastic container (Fisherbrand™) with 50 ml of double strength (2X) MacConkey broth  
148 (Thermo Fisher Scientific, Waltham, MA), mixed well and incubated at 35°C for 24h. Following  
149 incubation, a 10ul loopful of enrichment broth was plated onto MacConkey agar (BD BBL™,  
150 Sparks, MD) and incubated at 35°C for 24h. One colony demonstrating typical *E. coli*  
151 phenotypic morphology (pink, round) was picked from each plate and streaked for isolation  
152 onto blood agar plates (Thermo Fisher Scientific, Lenexa, KS) which were incubated at 35°C  
153 for 18-24h. Indole (BD BBL™, Sparks, MD) and oxidase (BD BBL™, Sparks, MD) quick tests  
154 were performed on suspected *E. coli* colonies; indole-positive, oxidase-negative colonies were  
155 confirmed to be *E. coli* by matrix-assisted laser desorption/ionization-time of flight (MALDI TOF;  
156 BioMerieux). All confirmed *E. coli* isolates were saved in cryovials containing *Brucella* broth  
157 (Thermo Fisher Scientific, Lenexa, KS) with 15% glycerol and subsequently stored at -80 °C  
158 for sequencing analysis.

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## 160 **DNA extraction and Whole Genome Sequencing**

161 DNA was extracted from each *E. coli* isolate using a modified version of the Qiagen DNeasy  
162 PowerLyzer microbial kit (Qiagen, Hilden, Germany). Using a 10ul loop, four passes of cells

163 were added to 300µl of PowerBead solution in a 1.5 ml microcentrifuge tube. Next, 50µl of SL  
164 solution was added to the PowerBead tubes which were immediately vortexed for 5 seconds  
165 to re-suspend. The bacterial suspension (300µl) was transferred to the PowerBead tubes which  
166 were placed in a bead mill (speed=6; time=1min) to homogenize. The PowerBead tubes were  
167 centrifuged (30secs at 17,000xg), and the supernatant was transferred to new 1.5 ml  
168 microcentrifuge tube (Qiagen, Hilden, Germany). Afterwards 100µl of IRS solution was added  
169 to the supernatant and vortexed briefly, followed by a 5 min incubation at 4°C. After incubation  
170 the tubes were centrifuged, and the supernatant again transferred to a new 1.5 ml  
171 microcentrifuge tube each containing 900µl of SB solution. The tubes were vortexed and 650µl  
172 of supernatant transferred to the spin column for spin washing (3 washes) and final elution.  
173 The Nanodrop 2000 Spectrophotometer was used to conduct a quality check for all the DNA  
174 samples. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA.  
175 A ratio of ~1.8 is generally accepted as “pure” for DNA. Subsequently the DNA concentration  
176 was quantified using Qubit 4.0 Fluorometer (ThermoFisher Scientific, Waltham, MA). DNA  
177 libraries of each sample were prepared for whole genome sequencing (WGS) using a Nextera  
178 XT kit (Illumina, San Diego, CA). Briefly, 0.3 ng/µl of DNA from each *E. coli* isolate was pooled  
179 together and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA) using 2 x  
180 250 or 2 x 300 paired-end approach. The raw paired end reads from the sequencer were  
181 demultiplexed and submitted to the NCBI database where they were assigned an accession  
182 number [21].

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## 184 **Assembly and assessment of genes**

185 *E. coli* genome sequences were assembled *de novo* using SPAdes version 3.15.4 via a web-  
186 based genome assembly service provided by the Bacterial and Viral Bioinformatics Resource



187 Center accessed online at <https://www.bv-brc.org/app/Assembly2>. The *in silico* analysis of  
188 acquired resistance genes and replicon typing for each *E. coli* isolate was conducted using the  
189 Mobile Element Finder tool (database version 1.0.2, 2020-06-09) accessed online via the  
190 Center for Genomic Epidemiology (CGE) website  
191 (<https://cge.food.dtu.dk/services/MobileElementFinder/>) [22]. The Mobile Element Finder tool  
192 interfaced with the ResFinder 4.1 tool to match individual genes for each *E. coli* isolate to an  
193 annotated resistance gene using a 90–100% identity, 60% minimum length, and 90% threshold  
194 [22]. PlasmidFinder 2.1 tool (database version 2023-01-18) set at a 95% minimum identity and  
195 60% coverage was used for replicon typing. We defined molecular MDR as the presence of  
196 resistance genes conferring AMR to three or more distinct classes of antimicrobials in the  
197 database.

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

200 *In silico* prediction of MLST was performed by submitting the assembled genome of an isolate  
201 to the *E. coli* PubMLST database (<https://pubmed.ncbi.nlm.nih.gov/30345391/>). The MLST 2.0  
202 (2022-11-14) tool on CGE website analyzed the contigs using previously described schemes  
203 by Achtman to assign sequence types (STs) based on allelic variations amongst seven  
204 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) [23]. *E. coli* isolates with  
205 identical sequences at all seven loci were assigned STs however those without perfect matches  
206 were usually identified as novel or unknown.

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## 208 **Determination of *E. coli* phylogroups and phylogeny**

209 The phylogenetic classification of the *E. coli* genomes was conducted using *in silico*  
210 ClermonTyping 1.4.1 tool as previously described [24]. The ClermonTyper web interface is

211 freely accessible at [http://clermonttyping.iame-research. center/](http://clermonttyping.iame-research.center/). The Nextflow workflow was  
212 used to map the *E. coli* whole genome fastq pair end reads to a reference genome as well as  
213 call variants which were used to generate a maximum likelihood phylogenetic tree  
214 ([https://gitlab.com/cgps/ghru/pipelines/snp\\_phylogeny](https://gitlab.com/cgps/ghru/pipelines/snp_phylogeny)). The clonal relationship between  
215 isolates was estimated using a pairwise single nucleotide polymorphism (SNP) analysis. The  
216 SNP analysis was used to determine how closely related the isolates were using ‘snp-dists’, a  
217 command line bioinformatics tool for transforming multiple DNA sequence alignment into a  
218 distance matrix (<https://github.com/tseemann/snp-dists>). Isolates that were less than 30 SNPs  
219 apart were related. The maximum likelihood phylogenetic tree was visualized using the  
220 interactive Tree of Life tool – iTOL version 6 (<http://itol.embl.de/itol.cgi>).

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## 222 **Data analyses**

223 Data were analyzed using R version 4.3 statistical software. The data were summarized by  
224 calculating frequencies and proportions. Pearson’s chi-squared test and Fisher’s exact test  
225 were used to compare MDR prevalence across retail meat types and significance was  
226 determined at a p-value  $\leq 0.05$ . The accession numbers for 117 paired end reads for the  
227 NARMS *E. coli* isolates collected in North Carolina by the NCSU CVM Thakur Molecular  
228 Epidemiology Laboratory for 2022 have been uploaded onto the National Center for  
229 Biotechnology Information (NCBI) database <https://www.ncbi.nlm.nih.gov> under bio projects  
230 accession number PRJNA292663. The additional data file for this study contains a list of  
231 accession numbers for individual Sequence Read Archive (SRA) for the *E. coli* isolates.

## 232 **Results**

233 From January through December 2022, a total of 312 meat samples were purchased from 48  
234 grocery stores in North Carolina, representing 8 counties. Of these samples, 69 (22.1%) were  
235 labeled with “no-antibiotic-ever”, 39 (12.5%) were labeled as organic, and 204 (65.3%) were  
236 identified as having no antibiotic use claim present. Of the 312 samples, 138 were positive for  
237 *E. coli* with an overall prevalence of 44.2%. Of these, 78 (56.5%) *E. coli* isolates were recovered  
238 from ground turkey, followed by chicken breast (n=27, 19.6%), chicken giblets (n=14, 10.1%)  
239 and pork chops (n=19, 13.8%). Regardless of meat type, the distribution of *E. coli* isolates  
240 across meat types was 15.2% (n=21) in organic products, 18.8% (n=26) in “no-antibiotic-ever”  
241 products and 65.9% (n=91) in products with “no claims”. The observed prevalence difference  
242 was not statistically significant across label claims ( $X^2=2.67$ ;  $p=0.26$ ). The prevalence of *E. coli*  
243 isolates differed by month of sampling with the highest prevalence observed in May (17/26;  
244 65.3%), followed by February and November (14/26; 53.8% each) while the least prevalence  
245 was observed in December (4/26; 15.3%) as shown in Figure 1. This observed difference  
246 between months was statistically significant ( $X^2=19.29$ ;  $p=0.05$ ). However, there was no  
247 significant association between *E. coli* prevalence and season of retail meat purchase from 138  
248 positive isolates ( $X^2=4.73$ ;  $p=0.19$ ). The highest prevalence (41/78; 52.6%) was observed in  
249 Spring and the lowest prevalence (29/78; 37.2%) was observed in Summer.

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252 **Figure 1: *Escherichia coli* occurrence in retail meat by month of sampling in North Carolina.**  
253 The highest *E. coli* occurrence was observed in May and the lowest in December 2022.

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255 Of all *E. coli* isolates, 84.8% (117/138) had good quality sequence data available for further  
256 characterization. Of these, 72 (61.5%) were recovered from ground turkey, 27 (23.1%) from  
257 chicken breast and 18 (15.4%) from pork chops. AMR genes were detected in 77.8% (91/117)  
258 of isolates, and 35.9% (42/117) were defined as MDR. The distribution of MDR *E. coli* isolates  
259 across retail meat type was 83.3% (35/42) for ground turkey, 9.5% (4/42) from chicken breast

260 and 7.1% (3/42) from pork chop, respectively. Sequencing analysis of AMR genes showed that  
261 the most prevalent resistance genes detected in the *E. coli* isolates from retail meats belonged  
262 to aminoglycosides (91.5%; 107/117), tetracyclines (59.8%; 70/117), beta-lactamases (31.6%;  
263 37/117), folate pathway antagonists (25.2%; 29/117) and quinolones (11.1%; 13/117) as shown  
264 in Table 1.

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

| AMR genes          | AMR class                     | Overall<br>n=117 (%) | Ground turkey<br>n=72 (%) | Chicken breast<br>n=27 (%) | Pork chop<br>n=18 (%) |
|--------------------|-------------------------------|----------------------|---------------------------|----------------------------|-----------------------|
| <i>aac(3)-Ild</i>  | Aminoglycosides               | 3 (2.6)              | 3 (4.2)                   | 0 (0)                      | 0 (0)                 |
| <i>aac(3)-IV</i>   |                               | 15 (12.8)            | 14 (19.4)                 | 1 (3.7)                    | 0 (0)                 |
| <i>aac(3)-VIa</i>  |                               | 5 (4.3)              | 3 (4.2)                   | 2 (7.4)                    | 0 (0)                 |
| <i>aadA1</i>       |                               | 15 (12.8)            | 9 (12.5)                  | 3 (11.1)                   | 3 (16.7)              |
| <i>aph(3'')-Ib</i> |                               | 29 (24.8)            | 24 (33.3)                 | 2 (7.4)                    | 3 (16.7)              |
| <i>aph(4)-Ia</i>   |                               | 15 (12.8)            | 15 (20.8)                 | 0 (0)                      | 0 (0)                 |
| <i>aph(6)-Id</i>   |                               | 25 (21.4)            | 20 (27.8)                 | 2 (7.4)                    | 3 (16.7)              |
| <i>blaCARB-2</i>   | Beta-lactamases               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>blaHERA-3</i>   |                               | 9 (7.7)              | 9 (12.5)                  | 0 (0)                      | 0 (0)                 |
| <i>blaOXA-1</i>    |                               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>blaTEM-1</i>    |                               | 24 (20.5)            | 20 (27.8)                 | 2 (7.4)                    | 2 (11.1)              |
| <i>blaTEM-141</i>  |                               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>blaTEM-206</i>  |                               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>dfrA1</i>       | Folate pathway<br>antagonists | 2 (1.7)              | 1 (1.4)                   | 1 (3.7)                    | 0 (0)                 |
| <i>dfrA14</i>      |                               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>dfrA15</i>      |                               | 1 (0.9)              | 0 (0)                     | 0 (0)                      | 1 (5.6)               |
| <i>sul1</i>        |                               | 11 (9.4)             | 8 (11.1)                  | 2 (7.4)                    | 1 (5.6)               |
| <i>sul2</i>        |                               | 14 (12.0)            | 11 (15.3)                 | 3 (11.1)                   | 0 (0)                 |
| <i>floR</i>        | Phenicols                     | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>fosA7</i>       | Phosphonic<br>antibiotics     | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>qacE</i>        | Quinolones                    | 8 (6.8)              | 5 (6.9)                   | 2 (7.4)                    | 1 (5.6)               |
| <i>qacL</i>        |                               | 2 (1.7)              | 2 (2.8)                   | 0 (0)                      | 0 (0)                 |
| <i>qnrB19</i>      |                               | 2 (1.7)              | 2 (2.8)                   | 0 (0)                      | 0 (0)                 |
| <i>qnrS1</i>       |                               | 1 (0.9)              | 1 (1.4)                   | 0 (0)                      | 0 (0)                 |
| <i>Inu(F)</i>      | Macrolides                    | 2 (1.7)              | 0 (0)                     | 0 (0)                      | 2 (11.1)              |
| <i>tetA</i>        | Tetracyclines                 | 29 (24.8)            | 23 (31.9)                 | 5 (18.5)                   | 1 (5.6)               |

|                       |  |           |           |          |          |
|-----------------------|--|-----------|-----------|----------|----------|
| <i>tetB</i>           |  | 41 (35.0) | 36 (50.0) | 2 (7.4)  | 3 (16.7) |
| <i>tet</i> gene (A&B) |  | 60 (51.3) | 49 (68.1) | 7 (25.9) | 4 (22.2) |

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

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307 **Table 2: Association of Multidrug Resistance (MDR) *Escherichia coli* in retail meat with**  
 308 **antibiotics use label claims**

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

309 \*Fisher's Exact Test

310

311 The MLST analysis showed that the 117 *E. coli* isolates belonged to 81 known sequence types  
312 (STs) with the most prevalent being ST117 (8.5%; 10/117), ST297 (5.1%; 6/117), ST58 (n=4;  
313 3.4%), and three isolates each (2.6%) for ST10, ST126, ST602, and ST1079. Numerous other  
314 STs were identified among the *E. coli* isolates with one or two isolates represented; ST131 and  
315 ST371 were not detected. The most prevalent MLST types detected in the isolates are shown  
316 in Figure 2.

317

318

319 **Figure 2: Multi-locus sequence types of *Escherichia coli* isolated from ground turkey, chicken breast, and**  
320 **pork chops in North Carolina, USA.** Each bar represents the various *E. coli* sequence types for isolates obtained  
321 from the different retail meat sources.

322

323

324 The 117 isolates in this study belonged to nine different phylogroups with the most prevalent  
325 phylogroup representing B1 (n=34; 29.1%) followed by phylogroup A (n=33; 28.2%),  
326 phylogroup B2 (n=16; 13.7%) and phylogroup G (n=11; 9.4%). Isolates with phylogroup B1  
327 were recovered from ground turkey (19/34; 55.9%), chicken breast (8/34; 23.5%) and pork  
328 chop (7/34; 20.6%) (Figure 3). Most isolates assigned ST297 (n=4) and ST58 (n=2) belonged  
329 to phylogroup A, while half of the isolates assigned ST117 (n=5) belonged to phylogroup B1.

330

331

332

333 **Figure 3: Phylogenetic classification of *Escherichia coli* isolates from retail meat types in North Carolina,**  
334 **2022.**

335

336 Thirty-four different replicon types were observed among *E. coli* isolates from retail meat. The  
337 most prevalent replicon types were IncFIB(AP001918) (n=73, 62.4%), Col(MG828) (n=50,  
338 42.4%) and IncFIC(FII) (n=43, 36.4%) as shown in Table 3. Thirteen replicon types were  
339 common to isolates from all retail meat sources: IncFIB(AP001918), IncFII, IncFIC(FII),  
340 IncFII(29), Col(MG828), Col156, ColpVC, Col4401, IncX1, IncFII(pCoo), IncFIA, IncI1, and

341 p0111. IncFIB(AP001918) was the most prevalent in all meat types: ground turkey (n = 51),  
 342 chicken breast (n=18) and pork chop (n= 3) followed by Col(MG828) detected in ground turkey  
 343 (n = 35), chicken breast (n=12) and pork chop (n= 3).

344

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

347

| 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)           |
| Incl1                  | 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)              |

348

349



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

357

358

359 **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             |

360

361

362 **Figure 4: SNP-based phylogeny of *Escherichia coli* isolates from retail meat in North Carolina, USA, 2022.**  
363 SNP-based maximum likelihood phylogeny of *E. coli* isolates visualized in interactive Tree of Life tool (iTOL). The  
364 tree was rooted in a reference *E. coli* strain K-12 MG1655. Clustering of isolates was found to be following the  
365 core genome and SNP-based phylogenies as clustering of isolates with the same sequence types and  
366 phylogroups was consistent. Shown for each isolate is the meat type, season, phylogroups, sequence types, AMR  
367 genes (brown) and plasmids (purple).

368

369 Seventeen isolates (14.5%) carried AMR genes on plasmid replicons identified on the same  
370 assembly scaffold, out of which 88.2% (15/17) were recovered from ground turkey and 11.8%  
371 (2/15) from chicken breast. The most prevalent replicon types were IncHI2 and IncHI2A

372 harboring aminoglycoside resistance gene *aph(3'')-Ib* in eight isolates (47.1%) and in  
 373 combination with *aph(6)-Id* in three of the eight isolates recovered from ground turkey. Other  
 374 AMR genes carried on plasmids include *bla*TEM-1A on IncI-1; *qacE* + *sul1* on IncFIC (FII);  
 375 *qnrB19* on Col440I amongst others (Table 5).

376

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

379

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

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

381

382

## 383 Discussion

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

393 In the present study, the highest prevalence of *E. coli* in the retail meat types was observed in  
394 May (spring). Our results show that there was no statistically significant association between  
395 the season of retail meat purchase and the detection of *E. coli*, although *E. coli* prevalence did  
396 differ by the month of sampling. The role of season on the presence of *E. coli* in retail meats is  
397 unclear as reports of another NARMS study in California found a prevalence of 36.7% in spring  
398 when compared to winter [17], while a similar study done in Canada found no definite seasonal  
399 trend [25]. The interplay between the effect of season and the prevalence of *E. coli* in retail  
400 meat is not fully understood [25].

401 Across all isolates from all retail meat sources, we found that there was a high proportion of  
402 genotypic AMR (77.8%) among *E. coli* and a moderate occurrence of MDR *E. coli* (35.9%); this  
403 prevalence is consistent with previous data collected from retail meat in the United States over  
404 the last decade [9, 17, 19, 26, 27]. The prevalence of resistance was highest in *E. coli* from  
405 retail ground turkey when compared to other meat types. This also is consistent with findings

406 from similar studies in Arizona which reported a high resistance prevalence of *E. coli* (90.7%)  
407 in retail turkey [19] and California reporting a prevalence of 70.4% [17]. This suggests there  
408 are differences in the management and production of turkey which may select for antimicrobial  
409 resistance or allow its persistence. It is important to note that producers in the United States  
410 are not required to make public reports on antimicrobial use data, hence it is difficult to link on-  
411 farm use of antimicrobials to development of AMR. Although between 2013 and 2017, the  
412 largest US turkey production corporations significantly reduced their overall use of  
413 antimicrobials attributed to the full implementation of FDA GFI #213, and improved  
414 antimicrobial stewardship amongst other factors [28].

415 The prevalence of genotypic resistance for ampicillin detected in *E. coli* from all meat types  
416 (31.6%; 37/117) was higher than the national average (11.6%; 54/466) for 2022; however, the  
417 prevalence of genotypic resistance for tetracycline was not different in *E. coli* from ground  
418 turkey (68.1%; 49/72) when compared to the national average of 62.5% (619/990). This was  
419 lower for chicken (25.9%; 7/27) and pork (22.2%; 4/18) when compared to the national average  
420 of 31.5% (147/466) and 50.4% (192/381) respectively [9]. In addition, to the ampicillin and  
421 tetracycline resistance genes, our results also show sulfonamide resistance genes and plasmid  
422 mediated quinolone resistance genes detected in retail meat. This finding aligns with past  
423 reports of the NARMS surveillance program [16–18].

424 Our data indicated that retail ground turkey may serve as an important source of MDR *E. coli*  
425 with the potential for transmission to humans if proper food handling practices are not used.  
426 However, there was no significant association between MDR *E. coli*, and the different  
427 antibiotics-use claims on the meat package labels. High resistance rates observed among *E.*  
428 *coli* isolates from turkey with “no antibiotic-use claims” may indicate that they were raised using  
429 conventional methods, however, this cannot be confirmed. This is consistent with the reports

430 of a similar study in Arizona, USA [19]. In conventional turkey production, while there may be  
431 a greater potential for antimicrobial use when compared to “no antibiotics ever” and “organic”  
432 production, we are not able to definitively determine the use.

433

434 The *E. coli* isolates from retail meat samples displayed diverse STs and phylogroups  
435 suggesting the possibility that the isolates had evolved overtime from different *E. coli* clones.  
436 The most prevalent known STs among the isolates were ST117 (recovered from chicken and  
437 turkey), ST297 and ST58 (both recovered from chicken breast). ST10 was also detected in  
438 isolates from turkey and pork. A study conducted in California, USA in 2018 identified ST117  
439 and ST10 in *E. coli* isolates from retail meat samples and humans [29]. Our results are  
440 consistent with reports of another related study that detected ST117 in *E. coli* recovered from  
441 commercial chicken and turkey [30] but at variance with others [30, 31] that reported ST58 and  
442 ST10 were dominant in turkey clinical *E. coli* isolates. The most dominant phylogroups in the  
443 present study were phylogroups A, B1 and B2 which was detected in isolates from all retail  
444 meat types and consistent with the literature [18, 32, 33].

445

446 The different replicon types along with the numerous *E. coli* STs in the present study highlight  
447 the diversity and complex nature of these indicator organisms. The most prevalent replicon  
448 types observed were IncFIB(AP001918), Col(MG828), IncFIC(FII), Col156, IncI1, p0111,  
449 IncFII, IncHI2, IncHI2A, Col440I and IncFIA. Seventeen isolates carried AMR genes on plasmid  
450 replicon identified on the same assembly scaffold. Replicon typing showed IncHI2 and IncHI2A  
451 harbored AMR gene *aph(3'')-Ib* + *aph(6)-Id* in some isolates recovered from ground turkey.  
452 Interestingly, the IncFIC(FII) plasmid was observed to harbor the *qacE* and *sul1* AMR gene on  
453 the assembly scaffold originating from one *E. coli* ST117 strain, recovered from retail chicken  
454 meat in the present study. This is of concern as ST117 is a known pathogenic *E. coli* lineage

455 that has been implicated in both human and animal diseases [29, 34]. Other AMR genes carried  
456 on plasmid contigs in our study include *bla*TEM-1A on IncI-1; *qacE* + *sul1* on IncFIC (FII);  
457 *qnrB19* on Col440I amongst others. Other studies have reported *E. coli* isolates from food  
458 animals carrying the plasmid mediated quinolone resistance gene *qnrB* on a Col440I replicon  
459 carrying plasmid contig supporting our study results [35, 36].

460

461 Studies have reported a high diversity between *E. coli* isolates recovered from retail meat types  
462 as evident by the wide variety of STs observed [29, 37]. The *E. coli* isolated from retail meat in  
463 the present study were genetically diverse hence we did not detect any clonal relationship  
464 between isolates from the different meat types. However, our results show that isolates from  
465 the same meat type i.e., ground turkey were found to be closely related within 20 SNPs.

466

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

## 476 **Conclusions**

477 In conclusion, we detected MDR *E. coli* from retail meat types in North Carolina. Our results  
478 showed that > 40% of the retail meats purchased from grocery stores were contaminated with

479 *E. coli*, and of these the vast majority were resistant to aminoglycosides (*aph(3'')*-*Ib* & *aph(6)*-  
480 *Id*), tetracycline (*tetA* & *tetB*) or ampicillin (*bla*TEM-1). The resistance prevalence was highest  
481 among *E. coli* isolates from turkey for most AMR genes detected. Our data indicated that  
482 ground turkey may serve as an important source of MDR *E. coli*. The isolates were diverse as  
483 only ten showed clonal relationships with a pairwise difference of  $\leq 20$  SNPs.

484 Ten of the *E. coli* isolated from retail meat had ST117 which is an emerging sequence type  
485 implicated as a human pathogen. It is important to emphasize that the bacteria isolated from  
486 retail meat samples in the present study were generic *E. coli* hence the risk of impacting  
487 negatively on human health is quite low. However, we cannot rule out the possibility of  
488 horizontal gene transfer between *E. coli* strains because our results show that plasmids, which  
489 are mobile genetic elements harbored some AMR genes which should be a source of concern  
490 for human health. Therefore, surveillance of these indicator bacteria strains should continue to  
491 serve as a warning for preventing the spread of AMR along the food chain. To further  
492 understand the transmission dynamics, additional studies are required especially due to the  
493 persistence of these acquired AMR genes and plasmid replicon types in different *E. coli* STs.

494

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500

## 501 **Authors' contributions**

502

503 MJ, ST and PC made substantial contributions to conception and design. EH collected data;  
504 EH, MA and CG performed laboratory analysis; EH and LH generated the whole genome  
505 sequence profile for all the isolates. MA performed bioinformatic analysis, interpreted the data  
506 and wrote the first draft of the manuscript. MJ, ST and PC revised article critically for important  
507 intellectual content. All authors read and approved the final manuscript.

508

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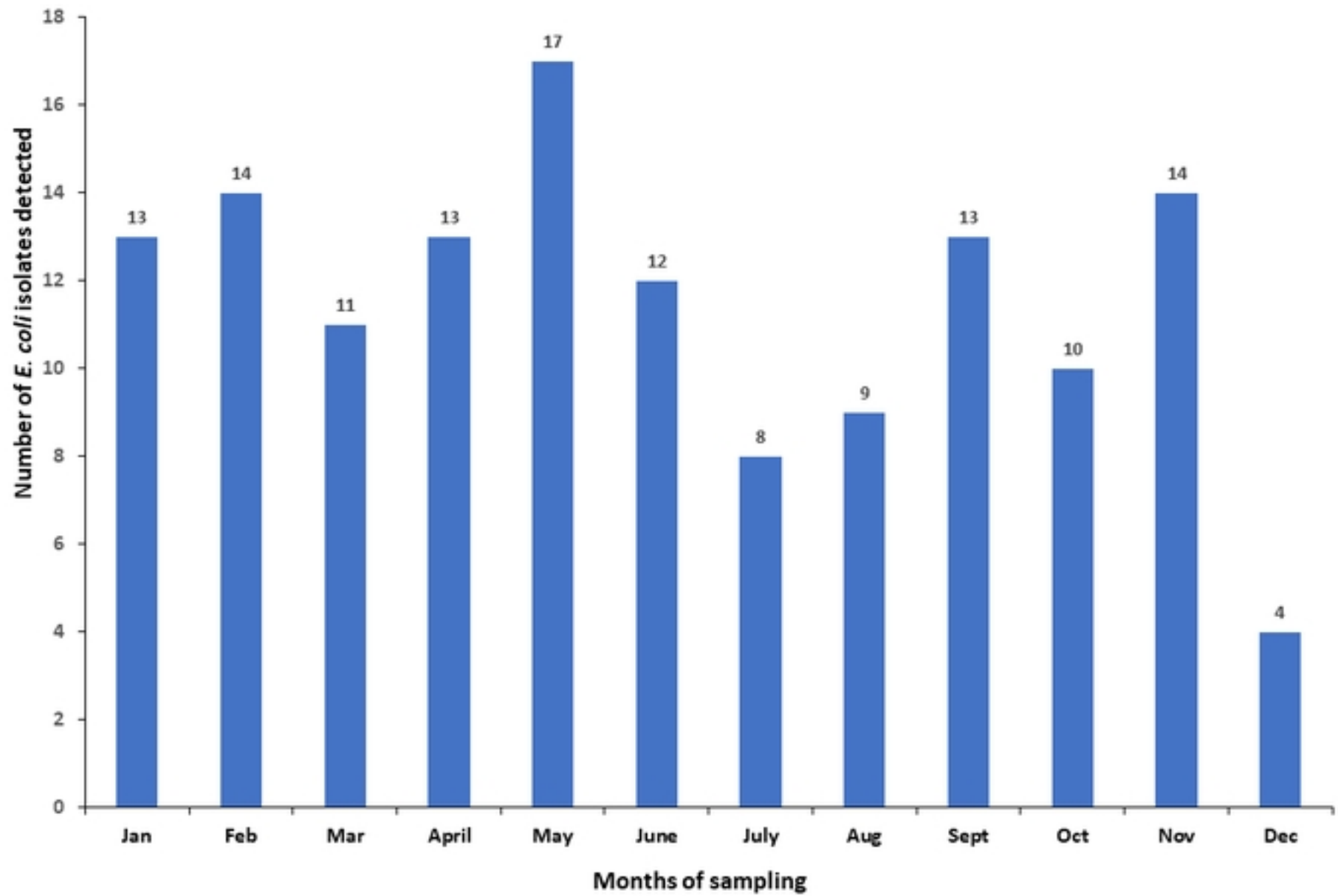


Figure 1

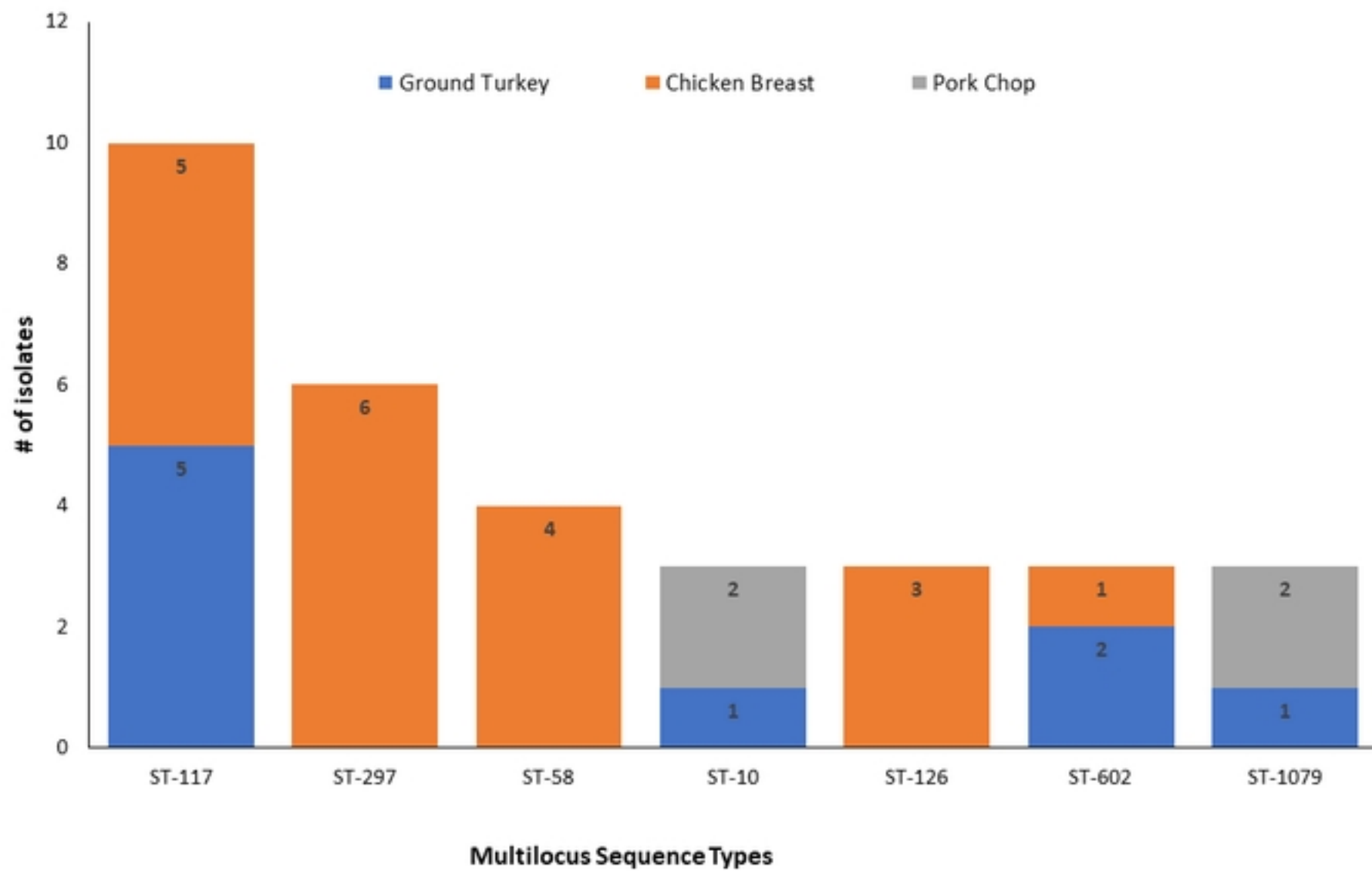


Figure 2

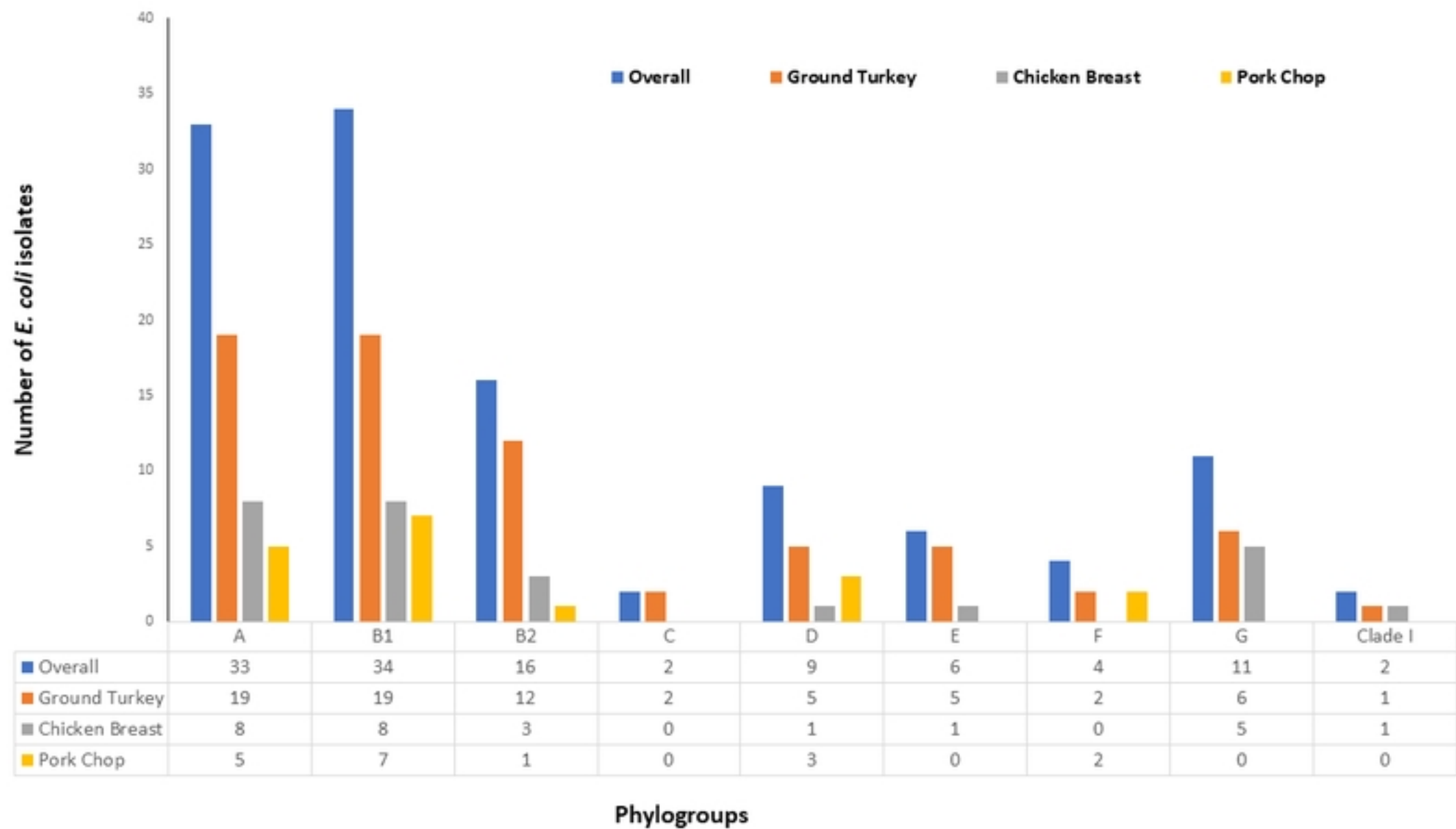


Figure 3

