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Turkey (*Meleagris gallopavo* f. *domestica*) Meat as a Potential Reservoir for Dissemination of Antimicrobial Resistant and Shigatoxigenic *Escherichia coli* Strains

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HIGHLIGHTS

- *Escherichia coli* was detected in 75.78% of turkey carcasses, with 52.7% of isolates being multidrug-resistant.
- A significant resistance to chloramphenicol (63.89%), nalidixic acid (59.72%), and florfenicol (56.94%) was detected.
- The virulence genes stx1 (4.17%), stx2 (1.39%), and eae (2.77%) were identified in the isolates.
- The *E. coli* isolates were assigned to phylotypes A, B1, C, D, and E, while 9.72% remained unclassified.
- The findings highlighted the public health risks associated with antibiotic-resistant STEC and EPEC strains in turkey meat.

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Abbreviations
AMR=Antimicrobial-Resistant
CLSI=Clinical and Laboratory Standards Institute
EHEC=Enterohemorrhagic *Escherichia coli*
EPEC=Enteropathogenic *Escherichia coli*
ESBL=Extended-Spectrum Beta-Lactamase
MDR=Multi-Drug Resistant
PCR=Polymerase Chain Reaction
STEC=Shiga Toxin-Producing *Escherichia Coli*

ABSTRACT

Background: Enteropathogenic and Shiga toxin-producing *Escherichia coli* (*E. coli*) are the two main pathotypes capable of causing serious human infections, especially when resistant to antibiotics.

Methods: In this work, 95 turkey carcasses were swabbed in slaughterhouse over four months (June–September 2023). Antibiotic resistance was evaluated by a disk diffusion method named Kirby–Bauer against nine antimicrobial agents. Three virulence genes including stx_1, stx_2, *eae*, three resistance genes including bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, and four phylogenetic markers (*arpA*, *chuA*, *yjaA*, *TspE4.C2*) were screened by Polymerase Chain Reaction (PCR) method. Data were analyzed using Microsoft Excel and SPSS (version 24), with the chi-square test at a significance level of $p \leq 0.05$.

Results: Out of 95 carcasses, 72 (75.78%) were *E. coli*-positive. Among the *E. coli* isolates, 63.89% were resistant to chloramphenicol, 59.72% to nalidixic acid, and 56.94% to florfenicol. One isolate, classified as extended-spectrum beta-lactamase positive, belonged to phylogroup D and showed simultaneous resistance to four antibiotics without harboring the resistance genes studied. Overall, 52.7% of the *E. coli* isolates were recognized as Multi-Drug Resistant (MDR). Profiles of resistance genes included bla_{TEM} (23.61%), bla_{TEM}/bla_{SHV} (2.77%), and $bla_{CTX\text{-}M}$ (1.38%). Virulence genes were detected in six isolates: stx_1 (4.17%), stx_2 (1.39%), and *eae* (2.77%). Phylogenetic analysis revealed five groups: A (19.44%), B1 (36.11%), C (5.55%), D (13.8%), and E (15.27%), while 9.72% remained unclassified.

Conclusion: The occurrence of *E. coli* isolates harboring virulence and antibiotic-resistance genes in turkey carcasses underscores serious public health risks.
The significant frequency of Multi-Drug Resistant (MDR) *E. coli* isolates highlights the need for improved monitoring and control measures throughout the food chain.

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Introduction

Turkey meat (*Meleagris gallopavo* f. *domestica*) is one the most consumed meat worldwide, following chicken meat (Connolly and Campbell, 2023). Meat products of turkey may be one of the putative reservoirs for several key foodborne pathogens, such as *Escherichia coli, Staphylococcus* spp., *Salmonella* spp., *Clostridium* spp., *Campylobacter* spp., *Listeria* spp., and *Arcobacter* spp. (Silva, Vidal and Junior, 2017). *E. coli* is of particular significance due to its predominant presence within the gastrointestinal microbiota in warm-blooded animals (Foster-Nyarko and Pallen, 2022).

E. coli is mainly commensal, but some strains exhibit pathogenicity due to distinct virulence factors (Pakbin, Brück and Rossen, 2021). Among the different pathotypes used to classify pathogenic *E. coli* strains, Shiga toxin-producing (STEC) and enteropathogenic *E. coli* (EPEC) are regarded as the most relevant to public health (Pakbin, Brück and Rossen, 2021). STECs produce two types of Shiga toxins named Stx1 and Stx2, which are encoded by *stx1* and *stx2* genes, whereas A distinctive feature of EPEC is its ability to cause attaching and effacing lesions, mediated by the outer membrane protein intimin, encoded by the *eae* gene (Pakbin, Brück and Rossen, 2021). The co-occurrence of intimin and stx_1 and/or stx_2 in *E. coli* strains defines the clinically significant pathotype called Enterohemorrhagic *E. coli* (EHEC; Zarei *et al*., 2021). *Stx* is a major virulence factor produced by STEC and EHEC, contributing to HC (hemorrhagic colitis) and HUS (Hemolytic Uremic Syndrome) (Freedman, Van De Kar and Tarr, 2023). Intimate adherence of bacteria to gut epithelial cells, causes disruption of the microvilli and formation of pedestal-like structures (Mare *et al*., 2021; Sokolovic *et al*., 2022).

One of the major challenges in infections caused by EPEC, STEC, and EHEC is the presence of antibiotic resistance in *E. coli* strains (Bouzari *et al*., 2018). The emergence of Antimicrobial-Resistant (AMR) *E. coli* in some food-producing animals, including poultry, has been increasingly reported worldwide (Van Boeckel *et al*., 2019). Studies have identified AMR *E. coli* isolates in meat products of turkey and other poultry, raising concerns about their potential foodborne transmission to humans (Khaitsa *et al*., 2008). These AMR strains may be resistant to fluoroquinolones and cephalosporins, which are essential for the treatment of severe human infections (Shrestha *et al*., 2022). The global health implications of AMR *E. coli* are significant, as these strains not only compromise treatment efficacy but also serve as reservoirs of resistance genes that may disseminate to other pathogenic/non-pathogenic bacteria (Martinez, 2009). Factors contributing to the emergence of AMR *E. coli* include improper antibiotic use, administration without prior antimicrobial susceptibility testing, and the utilization of counterfeit drugs; such practices exert selective pressure, favoring resistant bacteria while disadvantaging antibiotic-susceptible strains. Addressing this issue is crucial to public health, as it underscores the need for monitoring AMR *E. coli* prevalence in poultry and implementing more stringent antimicrobial stewardship practices (Endale, Mathewos and Abdeta, 2023).

E. coli populations are clonal and can be assigned to distinct phylogroups including A, B1, B2, D, C, E, F, G, and *Escherichia* cryptic clade I based on screening of four genetic markers: *arpA, TspE4.C2, yjaA* and *chuA* (Beghain *et al*., 2018). These phylogenetic groups reflect the virulence, evolutionary history, ecological niche and both genotypic/phenotypic characteristics of *E. coli* strains (Stoppe *et al*., 2017). Understanding the phylogroup is crucial, as certain groups are more commonly associated with virulence and antibiotic resistance features, thereby influencing the pathogenic potential and clinical outcomes of *E. coli* infections characteristics (Tenaillon *et al*., 2010).

Therefore, this study aims to investigate the prevalence of virulence genes and antimicrobial resistance factors among various phylogenetic groups of *E. coli* isolates from turkey meat. The results of this study may shed light on the contribution of turkey meat to the epidemiology of virulent and AMR *E. coli* strains.

Materials and methods

Collection of samples and isolation of E. coli

In total, 95 swab samples were collected from the carcasses of 95 turkeys in the slaughterhouse after chilling during four months (June to September 2023), before any secondary processing; Samples were taken by swabbing the interior surface of the turkey carcasses at the end of the slaughter line, prior to carcass cutting and packaging. A sterile swab was used to thoroughly swab the internal cavity of each carcass, moving from one end to the other 5 to 10 times to ensure sufficient sample collection. The swab was then placed into a transport medium for subsequent microbiological analysis. This standardized approach was aimed to ensure consistency and reproducibility across all samples. The sample size was estimated based on the formula:

$$Sample\ size = \frac{Z^2_{1-\frac{\alpha}{2}} \times p \times (1-p)}{d^2} = \frac{3.84 \times 0.4 \times 0.6}{0.01} = 92.16$$

Z value for the 95% confidence interval [error level (α): 0.05] = 1.96; $Z^2 = 3.84$

p [the projected proportion based on earlier research (Nwankwo *et al*., 2021)] = 0.4 (or 40%)

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$1 - p = 0.6$ (or 60%)

d (the error rate chosen by the investigators in this work) = 0.1 (or 10%); $d^2 = 0.01$

The swab samples were collected using aseptic techniques, stored in sterile tubes containing transport medium (Cary Blair; Himedia, India) under controlled temperature conditions (4°C), and immediately handled to laboratory mostly within 2 h and sometimes up to the 12 h. The swabs were transferred to the laboratory within 12 h. The samples were streaked on MacConkey agar (Merck, Germany) and incubated at 37 °C (Memmert, Germany) for 24 h. The lactose-positive colonies were biochemically confirmed by IMViC biochemical tests, including indole, methyl red, Voges-Proskauer, and citrate as *E. coli* strains (Markey *et al*., 2013). Then, one strain from each bird was considered for virulence, antimicrobial resistance, and phylogenetic assessments.

Phenotypic evaluation of the E. coli isolates

Antimicrobial resistance was determined in this work via the Kirby-Bauer disc diffusion method, performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021); the antibiotics used (Padtan Teb co., Iran) included ceftazidime, cefotaxime, cefotaxime clavulanate, ceftazidime clavulanate, florfenicol, chloramphenicol, enrofloxacin, nalidixic acid, and ciprofloxacin. The reference strain *E. coli* ATCC 25922 was used as the quality control strain, and the inhibition zone diameters were interpreted according to CLSI guidelines (CLSI, 2021). Isolates showing an increase of ≥ 5 mm in the inhibition zone for cefotaxime-clavulanate relative to cefotaxime alone, or for ceftazidime-clavulanate relative to ceftazidime alone, were considered Extended-Spectrum β-Lactamase-producing *E. coli* (ESBL; CLSI, 2021).

Polymerase Chain Reaction (PCR) for virulence, antimicrobial resistance, and phylogenetic genes

DNA extraction was carried out using boiling lysis method. In brief, *E. coli* colonies were suspended in 400 µl water (distilled and sterile) and heated at 98-100 °C for 10 min in boiling water. The lysates were then placed on (10 min) and centrifuged (13,000 × g; 1 min). The supernatant, containing the released DNA, was collected and used as the template for subsequent reactions. DNA yield and purity were evaluated with NanoDrop (BioTek Epoch, USA) by measuring absorbance at 260/280 nm.

The occurrence of the genes including stx_1, stx_2, and *eae* were screened (Paton and Paton, 2002) by PCR (Bio-Rad, USA). Sakaï strain of *E. coli* (*eae*+, stx_1+, and stx_2+) was used as a control$^+$ while *E. coli* strain MG1655 (*eae*−, stx_1−, and stx_2−) was employed as a control$^-$, sourced from Shahid Bahonar University of Kerman. Additionally, the *E. coli* strains were screened for $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} as antibiotic resistance genes (Roschanski *et al*., 2014) (Table 1).

Detection of four sequences (*arpA*, *chuA*, *yjaA,* and *TspE4.C2*) using PCR (multiplex) (Clermont *et al*., 2013) led to phylogenetic grouping of *E. coli* into 7 groups and some clades. Isolates that were *arpA* positive but *chuA*, *yjaA*, and *TspE4.C2* negative were allocated to phylogroup A, whereas those that were *arpA* positive, *chuA* and *yjaA* negative, but *TspE4.C2* positive were classified as B1. Phylogroup B2 included isolates that were *arpA* negative, *chuA* positive, and positive for at least one of *yjaA* or *TspE4.C2*. Isolates with the profile *arpA* positive, *chuA* negative, *yjaA* positive, and *TspE4.C2* negative were subjected to C-specific PCR; if this reaction was positive, they were assigned to C, otherwise they were reclassified as A. Strains that were *arpA* positive and *chuA* positive but *yjaA* negative (with *TspE4.C2* either negative or positive) were tested using E-specific primers; isolates yielding a positive result were designated as E, and those testing negative were placed in D. Isolates showing *arpA* positive, *chuA* positive, *yjaA* positive, and *TspE4.C2* negative were similarly screened with E-specific primers: E-positive strains were classified as E, whereas E-negative ones were assigned to *Escherichia* cryptic clade I and confirmed using cryptic clade-specific primers. Strains that were *arpA* negative, *chuA* positive, *yjaA* negative, and *TspE4.C2* negative were grouped as F, and isolates displaying *arpA* negative, *chuA* negative, *yjaA* positive, and *TspE4.C2* negative were assigned to *Escherichia* cryptic clade I or II after confirmation with cryptic clade-specific primers (Table 1). Other genotypes were considered as unknown phylogroups.

A 1.5% agarose gel in a horizontal electrophoresis unit was used to resolve the PCR products at 100 V for 45 minutes (Cleaver Scientific, United Kingdom) and then visualized by fluorescence staining using Greenview dye (Parstous Biotechnology, Iran) and gel documentation system (Vilber Lourmat co., Quantum software version ST4, France). Amplicon sizes were determined using two types of DNA marker including 50 bp and 100 bp (Parstous Biotechnology, Iran) as a reference.

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Table 1: Sequences of primers used in this study

Gene	Sequence (5'–3')	PCR condition	Product size(bp)	Reference
stx$_1$	F-ATAAATCGCCATTCGTTGACTAC R-AGAACGCCCACTGAGATCATC	35 cycles: 94°C (90 s), 60°C (90 s), 72°C (90 s)	348	(Paton and Paton, 2002)
stx$_2$	F-GGCACTGTCTCTCTGAAACTGCTCC R-TCGCCAGTTATCTGACATTCTG	35 cycles: 94°C (90 s), 60°C (90 s), 72°C (90 s)	584	(Paton and Paton, 2002)
eae	F-GACCCGGCACAAGCATAAGC R-CCACCTGCAGCAACAAGAGG	35 cycles: 94°C (90 s), 60°C (90 s), 72°C (90 s)	482	(Paton and Paton, 2002)
arpA	F-AACGCTATTCGCCAGCTTGC R-TCTCCCCATACCGTACGCTA	30 cycles: 94°C (5 s), 59°C (20 s) and 1 cycle 72°C (5 min)	400	(Clermont *et al*., 2013)
chuA	F-ATGGTACCGGACGAACCAAC R-TGCCGCCAGTACCAAAGACA	30 cycles: 94°C (5 s), 59°C (20 s) and 1 cycle 72°C (5 min)	288	(Clermont *et al*., 2013)
yjaA	F-CAAACGTGAAGTGTCAGGAG R-AATGCGTTCCTCAACCTGTG	30 cycles: 94°C (5 s), 59°C (20 s) and 1 cycle 72°C (5 min)	211	(Clermont *et al*., 2013)
TspE4.C2	F-CACTATTCGTAAGGTCATCC R-AGTTTATCGCTGCGGGTCGC	30 cycles: 94°C (5 s), 59°C (20 s) and 1 cycle 72°C (5 min)	152	(Clermont *et al*., 2013)
bla$_{TEM}$	Fa -GCGGAACCCCTATTTG Rb -ACCAATGCTTAATCAGTGAG	35 cycles: 95°C (30 s), 52°C (30 s), 72°C (60 s)	963	(Roschanski *et al*., 2014)
bla$_{SHV}$	F-TTATCTCCCTGTTAGCCACC R-GATTTGCTGATTTCGCTCGG	35 cycles: 95°C (30 s), 54°C (30 s), 72°C (30 s)	795	(Roschanski *et al*., 2014)
bla$_{CTX\text{-}M}$	F-CGATGTGCAGTACCAGTAA R-TTAGTGACCAGAATCAGCGG	35 cycles: 95°C (30 s), 60°C (30 s), 72°C (60 s)	585	(Roschanski *et al*., 2014)

Statistical analysis

To minimize potential confounding factors and biases, sampling was carried out over a 16-week period (4 months). During each sampling session, 5 to 10 samples were collected, and carcasses were selected in an alternating pattern (every other carcass) to ensure randomness and reduce selection bias. For each isolate, information on phylogroup status and antimicrobial resistance was compiled in Microsoft Excel 2016 and subsequently analyzed in SPSS version 24 (IBM) to obtain prevalence percentages through descriptive statistics. The chi-square test was applied to assess differences in frequencies and associations between variables, and a p-value of ≤ 0.05 was taken as the threshold for statistical significance.

Results and discussion

Prevalence of E. coli strains

E. coli was detected in 72 out of 95 turkey carcasses (75.78%). The presence of *E. coli* on turkey carcasses suggests shortcomings in slaughterhouse hygiene, including possible fecal cross-contamination or improper carcass handling. Such contamination represents a potential public health risk. *E. coli* may contribute substantially to the spread of virulence factors and antimicrobial resistance determinants.

Prevalence of phenotypic antimicrobial resistance

Antimicrobial resistance to seven widely used antibiotics was investigated; these antibiotics belonged to three different classes and were tested using the disc diffusion method. For amphenicols, 46 (63.89%) isolates showed resistance against chloramphenicol, and 41 (56.94%) isolates were resistant to florfenicol. For quinolones, resistance against enrofloxacin, nalidixic acid, and ciprofloxacin was detected in 23 (31.94%), 43 (59.72%), and 17 (23.61%) isolates, respectively. The lowest level of resistance was identified to β-lactams ($p \leq 0.05$); only four strains were resistant against cefotaxime (5.56%), one was ceftazidime-resistant (1.38%), and only one isolate was ESBL-positive (1.38%) (Table 2). No differences were significantly observed in the frequency of antibiotic resistance among the phylogenetic groups ($p > 0.05$).

Table 2: Prevalence of antimicrobial resistance phenotype among *Escherichia coli* isolates

Antimicrobial family	Antimicrobial resistance phenotype	Number	Percentage	95% CI
β-lactams				
	Cefotaxime	4	5.56	0.26-10.85%
	Ceftazidime	1	1.38	0-4.09%
	Extended-Spectrum Beta-Lactamase-positive	1	1.38	0-4.09%
Amphenicols				
	Florfenicol	41	56.94	45.51-68.38%
	Chloramphenicol	46	63.89	52.79-74.98%
Quinolones				
	Enrofloxacin	23	31.94	21.17-42.71%
	Nalidixic acid	43	59.72	48.39-71.05%
	Ciprofloxacin	17	23.61	13.80-33.42%

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The elevated levels of resistance to chloramphenicol, nalidixic acid, and florfenicol found in this study are consistent with previous findings in *E. coli* strains recovered from turkey meat and intestinal contents in Iran (Gholami-Ahangaran *et al*., 2021); furthermore, the low levels of resistance to cefotaxime and ceftazidime observed in our study agreed with those reported previously in Germany, (Kaesbohrer *et al*., 2012), Poland (Wasyl *et al*., 2013), and Iran (Mousavi, Rahimi and Shakerian, 2020).

Lowest resistance level was against the β-lactams. The rate of antibiotic resistance for cefotaxime in this study was lower than study in Egypt (AbdelRahman *et al*., 2020; Moawad *et al*., 2018; Younis, Awad, and Mohamed, 2017), China (Wang *et al*., 2021), and India (Sebastian *et al*., 2021); only one isolate of our study was ESBL-positive. The lower levels of resistance against β-lactams in turkey meat, compared to florfenicol and chloramphenicol, may reflect differences in antibiotic usage practices in turkey production.

Antibiotic usage in veterinary medicine is a widespread approach either as a preventive measure or as additives to promote growth in livestock (Vázquez-Villanueva *et al*., 2023). Unfortunately, this has resulted in the appearance of bacterial strains that exhibit resistance to multiple antibiotics, including florfenicol, chloramphenicol, cefotaxime, and ceftazidime. The present study demonstrates a considerable presence of AMR strains, suggesting a lack of adherence to recommended guidelines and an overuse of antibiotics.

Resistance patterns and their distribution among phylogroups are shown in Table 3 and Figure 1. Twenty antimicrobial resistance profiles were observed in which florfenicol-chloramphenicol was the most prevalent AMR profile (12 isolates; 16.67%). Thirty-eight (52.7%) isolates were recognized as Multi-Drug Resistant (MDR; a strain, resistant to at least one antibiotic from three different antimicrobial classes); this finding is comparable to the results reported from the USA (Davis *et al*., 2018). Six strains were susceptible to all tested antimicrobials. The most common resistance pattern was related to FF, C profile (12 isolates, 16.69%). In Egypt, a work on *E. coli* strains isolated from turkey showed 100% of the isolates were MDR (Eid and Samir, 2019). The global dissemination of MDR strains as a consequence of excessive and uncontrolled use of antimicrobials has caused a serious problem, indicating the high prevalence of MDR isolates among poultry farms.

Detection of β-lactamase genes

The most frequent antimicrobial resistance genotypic pattern was bla_{TEM} (17 isolates; 23.61%), followed by bla_{TEM}/bla_{SHV} (2 isolates; 2.77%), and $bla_{CTX\text{-}M}$ (1 isolate; 1.38%). Fifty-two isolates (72.22%) possessed none of the screened antimicrobial resistance genes. The phylogenetic distribution pattern of the positive isolates is revealed in Table 4.

Table 3: Phenotypic antimicrobial resistance profiles and their distribution pattern among phylo-groups

Antimicrobial resistance profiles	Phylo-group (no.)						
	A	B1	C	D	E	U	No. (%)
C		1				2	3 (4.17)
FF	1	1		1	1		4 (5.57)
NA		1	2	3	1		7 (9.73)
CTX		1					1 (1.38)
FF, C	5	3			1	3	12 (16.69)
C, NA						1	1 (1.38)
FF, NA		1					1 (1.38)
C, CTX	1						1 (1.38)
NA, CIP			1				1 (1.38)
FF, CTX					1		1 (1.38)
NA, NFX		1			1		2 (2.77)
FF, C, NA		3		3	2		8 (11.12)
FF, C, CIP				1			1 (1.38)
C, NA, NFX		1					1 (1.38)
NA, NFX, CIP	1			1	1		3 (4.17)
FF, C, NA, CIP		1					1 (1.38)
FF, C, NA, NFX	2	3			1		6 (8.34)
C, NA, NFX, CIP	1	1		1*		1	4 (5.57)
C, NA, CTX, CAZ	1						1 (1.38)
FF, C, NA, NFX, CIP	2	4			1		7 (9.73)
Non-resistant isolates	1	4			1		6 (8.34)
Total (%)	15 (20.83)	26 (36.11)	3 (4.17)	10 (13.89)	11 (15.28)	7 (9.72)	72 (100)

*This strain is the only Extended-Spectrum Beta-Lactamase-producing *Escherichia coli* isolate.
C=chloramphenicol; CAZ=ceftazidime; CIP=ciprofloxacin; CTX=Cefotaxime; FF=florfenicol; NA=nalidixic acid; NFX=enrofloxacin

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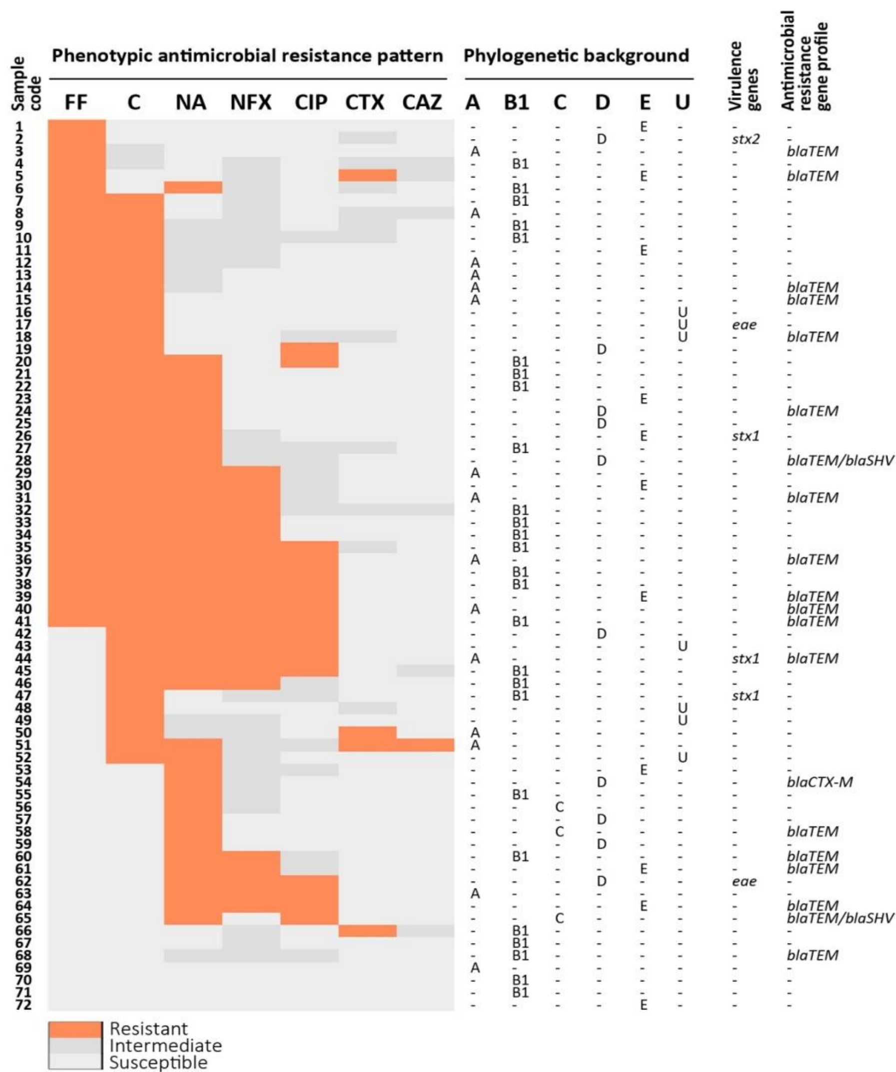

Figure 1: Heat map of phenotypic antimicrobial resistance patterns, phylogenetic background, virulence genes, and antimicrobial resistance genes.
C=chloramphenicol; CAZ=ceftazidime; CIP=ciprofloxacin; CTX=Cefotaxime; FF=florfenicol; NA=nalidixic acid; NFX=enrofloxacin

Table 4: Prevalence of antimicrobial resistance genotype and distribution pattern of phylogenetic groups among *Escherichia coli* isolates

Variables	Phylo-group						No. (%)	95% confidence interval
	A	B1	C	D	E	U		
bla_{TEM}	7	3	1	1	4	1	17 (23.61)	13.8%-33.42%
bla_{TEM}/bla_{SHV}			1		1		2 (2.77)	0%-6.57%
bla_{CTX-M}				1			1 (1.38)	0%-4.09%
No resistance gene	8	23	1	8	6	6	52 (72.22)	61.88%-82.57%
Total	15	26	3	10	11	7	72 (100)	-

Higher frequency of *bla* gene-positive strains in poultry and turkey has been reported in Egypt (AbdelRahman *et al*., 2020), Ghana (Eibach *et al*., 2018), Türkiye (Baran, Adıgüzel and Yüksel, 2020), and Brazil (Hoepers *et al*., 2018). These studies are relevant as they demonstrate the widespread occurrence of *bla* genes in poultry across diverse geographic regions. The present study's results on the bla_{CTX-M} closely align with finding from Canada (Sheikh *et al*., 2012) and Egypt (Moawad *et al*., 2018).

It is also important to recognize the potential for horizontal transfer of ESBL genes to other bacteria. Such transfer events may promote the dissemination of resistance features to otherwise susceptible bacterial populations, thereby increasing the likelihood of therapeutic failure and making infection control more difficult.

Prevalence of virulence genes

In the current study, six strains carried at least one of the

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three virulence genes under investigation; *stx_1* (3 isolates; 4.17%), *stx_2* (1 isolate; 1.39%) and *eae* (2 isolates; 2.77%). No virulence genes were detected in 66 isolates (91.67%). Details are presented in table 5.

Furthermore, six strains harbored at least one of the three virulence genes analyzed, which are known to be associated with STEC, EPEC, and EHEC pathotypes; the prevalence of VG-positives was less than 5%. Comparable frequencies of *stx_1*, *stx_2*, and *eae* were found by Mousavi, Rahimi and Shakerian (2020) in Iran. However, some studies in Saudi Arabia (Hessain *et al*., 2015), Austria (Mayrhofer *et al*., 2004), and Canada (Bohaychuk *et al*., 2006) did not detect virulence genes related to STEC, EPEC, and EHEC.

Table 5: Prevalence of virulence gene and distribution pattern of phylogenetic groups among *Escherichia coli* isolates

Variables	Phylo-group						No. (%)	95% confidence interval
	A	B1	C	D	E	U		
eae				1		1	2 (2.77)	0%-6.57%
stx_1	1	1			1		3 (4.17)	0%-8.78%
stx_2				1			1 (1.39)	0%-4.09%
No virulence gene	14	25	3	8	10	6	66 (91.67)	82.74%-96.88%
Total	15	26	3	10	11	7	72 (100)	-

The variation in sample size between studies may be one reason for the observed differences in findings. The lower prevalence of the virulence genes in turkey meat compared to other food sources such as beef can be attributed to biological and husbandry-related factors. It is well-established that ruminants, rather than birds, serve as the primary reservoirs of STEC. Therefore, the presence of these virulence genes is generally higher in ruminant-derived products. However, in traditional turkey farming systems, especially where turkeys are raised in close proximity to ruminants, the likelihood of STEC contamination may increase due to potential cross-contamination or environmental exposure.

Available evidence suggests that food animals might function as a reservoir for human infection. Also, retail meats could potentially serve as a vehicle for the transmission of these strains. Although the frequency of pathogenic *E. coli* isolates in turkey carcasses is low, their potential role in spreading virulent strains should not be overlooked, especially considering vulnerable populations such as immunocompromised individuals, for whom even minimal exposure may pose significant health risks.

Prevalence of phylogenetic groups

Totally, among the 72 strains, seven phylogroups including A, B1, B2, C, D, E, and F were identified, while some isolates remained unassigned and were categorized as unknown (U); from high to low prevalence, 26 isolates (36.11%) belonged to B1, 14 isolates (19.44%) to A, 11 (15.27%) isolates to E, 10 isolates (13.8%) to D, 4 isolates (5.55%) to C, and 7 isolates (9.72%) didn't belong to any of the phylogenetic groups (U) (Table 3-5). A heat map of phenotypic antimicrobial resistance patterns and associated phylogenetic background, virulence genes, and antimicrobial resistance genotypic profile has been provided in Figure 1.

The dominance of B1 in our study is consistent with another work in Türkiye (Baran, Adıgüzel and Yüksel, 2020). The most prevalent phylotypes in China (Wang *et al*., 2021), Spain (Egea *et al*., 2012), and Iran (Asadi *et al*., 2018) have been related to phylotype A. Various variables, for example body mass, age, diet, species, sex, type of nutrition, types of pollution, environment (geographic location and climate), bacteria (pathogenicity and resistance), method, sample size, and study time, account for differences in phylotypes across studies.

Most positive strains for β-lactam were found in A and B1, the phylogroups known as commensal phylogroups of *E. coli*. Therefore, commensal strains may represent a potential threat to public health because of their role in the transmission of antibiotic resistance. among different hosts and pathogens. Strains classified within phylogroup D were all negative for virulence genes. This finding is interesting because, in many studies, D has been introduced as a phylogroup related to pathogenic strains of *E. coli*. Although B2 and D are traditionally considered more pathogenic in humans and other hosts, some studies have reported that strains belonging to these groups may exhibit lower virulence potential. This observation suggests that phylogenetic grouping alone may not fully predict the pathogenicity of *E. coli* bacterium.

Phylogroups of *E. coli* may be associated with distinct antibiotic resistance patterns; however, this relationship is complex and influenced by multiple factors. The transfer of genes encoding resistance factors through mobile genetic elements can modify resistance patterns among different phylogroups (Xie, Ogura and Suzuki, 2022).

Conclusion

The findings of this study indicate a high prevalence of *E. coli* in turkey carcasses. turkey carcasses, with a substantial proportion of isolates identified as multidrug-resistant and harboring the genetic determinants related to virulence and antibiotic resistance. Occurrence of virulent and AMR *E. coli* isolates from turkey meat highlights

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significant public health concerns, particularly in relation to food safety and antibiotic stewardship.

The results highlight the attention to regular surveillance of antimicrobial resistance patterns in poultry meat, as well as the implementation of effective hygiene practices throughout the slaughtering process. Improved slaughterhouse hygiene, personnel training, and adherence to international standards, including Hazard Analysis and Critical Control Points (HACCP), are essential for minimizing microbial contamination.

Given the limitations of the present study, small sample size and the selection of only one isolate per bird, further research is recommended to better capture the heterogeneity and dynamics of *E. coli* bacterium in poultry.

Future studies should involve larger and geographically diverse sampling, comparison with other poultry species, and an expanded panel of virulence genes to gain a better understanding of the pathogenic ability and antimicrobial resistance profiles of *E. coli* in turkey meat.

Author contributions

M.B. conceptualized and administrated the project and wrote, reviewed and edited the manuscript; R.G. supervised the research and reviewed the manuscript; Z.A., P.M., M.H.A., and M.J. performed the sampling, laboratory procedures, and data analysis. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable.

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