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Molecular Characterization of Multidrug-Resistant *Escherichia coli* Isolated from Beef and Chicken Meat Products

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Abstract

Background: Foodborne pathogenic *Escherichia coli* is a major public health concern due to its frequent association with meat and meat products and its potential to harbor virulence factors and antimicrobial resistance determinants. **Objective:** This study aimed to determine the prevalence, virulence gene profiles, and antimicrobial resistance characteristics of *E. coli* isolates obtained from beef and chicken meat products. **Methods:** A total of 200 beef and chicken meat product samples were collected from retail markets in Samsun, Türkiye. Isolation of *E. coli* was performed using conventional culture-based methods, and PCR targeting the *uspA* gene was used for molecular confirmation. The presence of virulence genes (*stx1, stx2, eae,* and *hlyA*) was investigated by PCR. Antimicrobial susceptibility testing was conducted using the disk diffusion method, and multidrug resistance (MDR) and multiple antibiotic resistance (MAR) indices were evaluated. **Results:** Among the 200 samples analyzed, 80 (40%) were positive for *E. coli*, including 38 (38%) beef and 42 (42%) chicken meat samples. A total of 185 *E. coli* isolates were recovered and confirmed by PCR. Virulence gene analysis showed that *stx2* was the most prevalent gene (51.4%), followed by *eae* (37.3%), *hlyA* (13.0%), and *stx1* (6.5%). Antimicrobial susceptibility testing demonstrated high resistance rates to tetracycline (69.7%), ampicillin (58.3%), trimethoprim-sulfamethoxazole (48.1%), streptomycin (40.5%), nalidixic acid (40.0%), chloramphenicol (40.0%), and ciprofloxacin (34.1%). In contrast, the lowest resistance rates were observed for imipenem (2.1%), amoxicillin-clavulanate (4.8%), and amikacin (7.5%). Moreover, 126 isolates (68.1%) were identified as MDR, exhibiting resistance to at least three antimicrobial agents. The MAR index ranged from 0.0 to 1.0. **Conclusions:** The coexistence of virulence-associated genes and high antimicrobial resistance rates among *E. coli* isolates from meat products indicates a potential public health risk. These findings highlight the importance of continuous monitoring of pathogenic and antimicrobial-resistant *E. coli* throughout the food production chain.

Keywords: *Escherichia coli*; virulence genes; antimicrobial resistance; multidrug resistance; beef meat; chicken meat

1. Introduction

Meat is an important component of the human diet due to its high nutritional value, particularly its rich content of proteins, vitamins, and minerals [1]. However, its high water activity, nutrient availability, and near-neutral pH provide favorable conditions for microbial growth, making meat and meat products highly susceptible to spoilage and microbial contamination. During slaughtering, processing, transportation, packaging, and distribution, cross-contamination may occur at multiple stages, thereby increasing the microbial load and compromising food safety [2]. For this reason, meat and meat products are considered an important public health concern with respect to several pathogenic microorganisms, particularly *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli*, and *Listeria monocytogenes* [3,4].

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, non-spore-forming bacterium commonly found in the intestinal microbiota of humans and warm-blooded animals [5]. Contamination of meat products may occur through fecal material during slaughter and processing or through environmental sources and handling practices. Therefore, *E. coli* is considered both an indicator organism and an important foodborne pathogen associated with meat and meat products [4,6]. Raw or undercooked meat products, particularly minced meat and hamburger products, play a major role in the transmission of pathogenic *E. coli* strains to humans [7]. In addition, certain pathogenic strains, especially Shiga toxin-producing *E. coli* (STEC), are capable of causing severe gastrointestinal diseases, including hemorrhagic colitis and hemolytic uremic syndrome (HUS), which may result in life-threatening complications [8].

The pathogenicity of *E. coli* is largely associated with specific virulence factors, particularly the Shiga toxin genes (*stx1* and *stx2*), the adhesion-related intimin gene (*eae*), and the hemolysin gene (*hlyA*) [9,10]. The *stx1* and *stx2* genes encode Shiga toxins that inhibit protein synthesis in host cells and contribute to severe clinical manifestations such as hemorrhagic colitis and HUS [11,12]. The *eae* gene encodes intimin, an outer membrane adhesion protein responsible for intimate attachment to intestinal epithelial cells and the formation of attaching-and-effacing lesions [9]. The *hlyA* gene encodes α-hemolysin, an RTX (repeats-in-toxin) family toxin associated with membrane damage, cell lysis, and increased disease severity [13]. Detection of these virulence-associated genes in meat products is therefore considered an important indicator of potential public health risk.

In recent years, antimicrobial resistance (AMR) has emerged as a major global public health concern, threatening the effectiveness of antimicrobial agents used in both human and veterinary medicine. The extensive and often uncontrolled use of antimicrobials in food-producing animals has contributed substantially to the emergence and dissemination of resistant bacteria throughout the food chain [14,15]. Among foodborne pathogens, *E. coli* is considered a key indicator organism for AMR surveillance because of its widespread distribution and its ability to acquire and transfer resistance determinants through mobile genetic elements. In particular, multidrug-resistant (MDR) *E. coli* strains have increasingly been reported in retail meat products worldwide, posing a considerable risk to consumers [16–18].

The present study aimed to determine the occurrence of *E. coli* in beef and chicken meat products, characterize the prevalence of major virulence genes (*stx1*, *stx2*, *eae*, and *hlyA*), and evaluate the antimicrobial resistance profiles of the isolates. These findings may contribute to ongoing surveillance programs and support One Health strategies aimed at limiting the dissemination of antimicrobial-resistant foodborne pathogens throughout the food production chain.

2. Results

2.1. Prevalence and Virulence Gene Profiles of E. coli Isolates

E. coli was detected in 80 (40%) of the 200 samples analyzed. Of these, 38/100 (38%) beef meat product samples and 42/100 (42%) chicken meat product samples were positive for *E. coli*. Among beef meat products, the highest contamination rate was observed in hamburger samples (13/20, 65%), followed by minced beef (11/20, 55%) and meatballs (8/20, 40%), whereas diced beef and sausage showed lower contamination levels (3/20, 15% each). Among chicken meat products, chicken breast (15/20, 75%) and chicken drumstick (13/20, 65%) exhibited the highest contamination rates, followed by chicken wing (11/20, 55%) and chicken sausage (3/20, 15%), while no *E. coli* was detected in chicken burger samples.

A total of 185 *E. coli* isolates were recovered, and all isolates were confirmed by PCR targeting the *uspA* gene. The expected amplicon sizes were 884 bp for *uspA*, 347 bp for *stx1*, 589 bp for *stx2*, 890 bp for *eae*, and 165 bp for *hlyA* (Figures 1–3). Virulence gene analysis revealed that 12/185 (6.5%) isolates carried *stx1*, 95/185 (51.4%) carried *stx2*, 69/185 (37.3%) carried *eae*, and 24/185 (13.0%) carried *hlyA*. Among the investigated virulence genes, *stx2* was the most prevalent, followed by *eae*, *hlyA*, and *stx1*. The prevalence and virulence profiles of the *E. coli* isolates are summarized in Table 1.

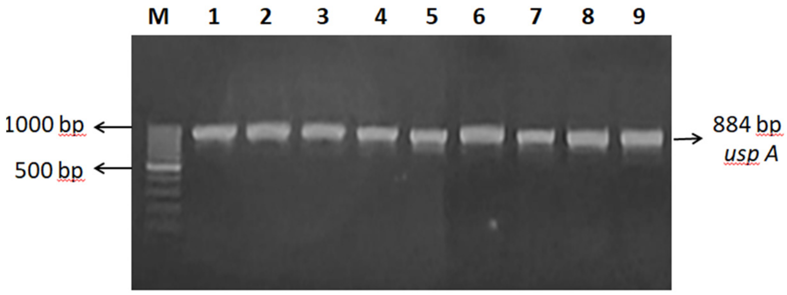

Figure 1. PCR amplification of the *uspA* gene in *E. coli* isolates. M: 100 bp DNA marker; Lane 1: positive control (*E. coli* ATCC 43888); Lanes 2–9: *uspA*- positive *E. coli* isolates.

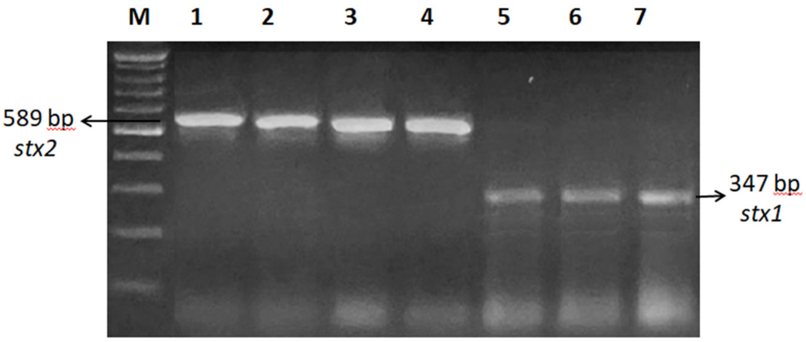

Figure 2. PCR amplification of the *stx1* and *stx2* genes in *E. coli* isolates. M: 100 bp DNA marker; Lane 1: positive control (*E.coli* ATCC 43889, *stx2*+); Lanes 2–4: *stx2*-positive *E. coli* isolates; Lane 5: positive control (*E.coli* ATCC 43890, *stx1*+); Lanes 6–7: *stx1*-positive *E. coli* isolates.

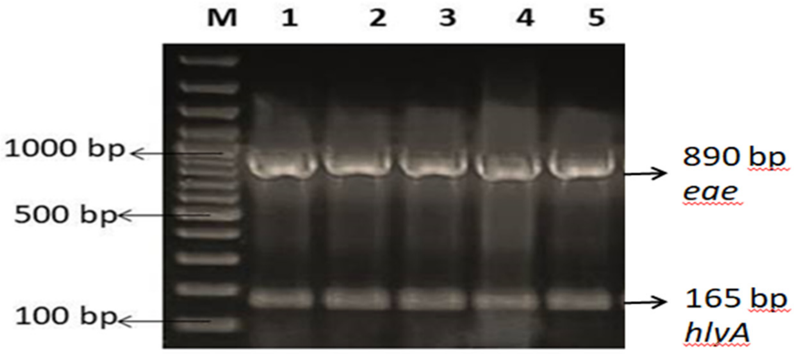

Figure 3. PCR amplification of the *eae* and *hlyA* genes in *E. coli* isolates. M: 100 bp DNA marker; Lane 1: positive control (*E. coli* O157:H7 ATCC 43888, *hlyA*+, *eae*+); Lanes 2–5: *eae and hlyA*-positive *E. coli* isolates.

Table 1. Distribution of *E. coli* occurrence and virulence genes in beef and chicken meat products.

		E. coli (*uspA* gene)			Virulence genes of *E. coli*			
	Sample type	**n**	**Sample**	**Isolate**	***stx1***	***stx2***	***eae***	***hlyA***
Beef meat	Diced beef	20	3 (15%)	11	-	3	3	1
	Minced beef	20	11 (55%)	21	5	10	7	6
	Meatball	20	8 (40%)	20	1	15	7	6

products	Hamburger	20	13 (65%)	34	2	12	14	1
	Sausage	20	3 (15%)	7	1	7	ND	6
	Total	100	38 (38%)	93	9	47	31	20
Chicken meat products	Breast	20	15 (75%)	30	-	16	14	1
	Drumstick	20	13 (65%)	33	1	16	12	1
	Wing	20	11 (55%)	26	2	14	9	2
	Sausage	20	3 (15%)	3	ND	2	3	ND
	Burger	20	ND	ND	ND	ND	ND	ND
	Total	100	42 (42%)	92	3	48	38	4
	Ovarall total	200	80 (40%)	185	12 (6.5%)	95 (51.4%)	69 (37.3%)	24 (13.0%)

ND: non detected.

2.2. Multidrug Resistance of *E. coli* Isolates

The antimicrobial resistance profiles and multiple antibiotic resistance (MAR) index values of *E. coli* isolates obtained from beef and chicken products are presented in Tables 2 and 3. The MAR index was calculated using the formula MAR = a/b, where *a* represents the number of antibiotics to which an isolate was resistant and *b* represents the total number of antibiotics tested. The MAR index values ranged from 0.00 to 1.00, indicating marked variation in antimicrobial resistance patterns among the isolates.

Antimicrobial susceptibility analysis revealed high resistance rates to tetracycline (69.7%), ampicillin (58.3%), trimethoprim-sulfamethoxazole (48.1%), streptomycin (40.5%), nalidixic acid (40.0%), chloramphenicol (40.0%), and ciprofloxacin (34.1%). In contrast, lower resistance rates were observed for imipenem (2.1%), amoxicillin–clavulanate (4.8%), and amikacin (7.5%). Of the 185 isolates tested, 72 (38.9%) exhibited a MAR index ≤ 0.2, whereas 113 (61.1%) showed a MAR index > 0.2, indicating possible exposure to high-risk contamination sources associated with frequent antimicrobial use. Overall, 126 isolates (68.1%) were classified as multidrug-resistant (MDR), defined as resistance to at least three antimicrobial agents. Resistance pattern analysis demonstrated a wide distribution of resistance phenotypes among the isolates. The proportions of isolates resistant to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 16 antibiotics were 17.2%, 9.7%, 4.8%, 7.0%, 9.1%, 15.1%, 8.1%, 5.9%, 5.4%, 7.5%, 4.8%, 3.2%, 1.1%, and 0.5%, respectively. Notably, a considerable proportion of isolates exhibited resistance to six or more antibiotics, and one isolate showed resistance to all tested antimicrobials.

Table 2. Antibiotic resistance profiles of *E. coli* isolates (n = 185).

		No. (%) of *E. coli* isolates		
Antibiotics	Concentration	Resistant	Intermediate	Susceptible
Amikacin	(AK, 30 µg)	14 (7.5%)	1 (0.5%)	170 (91.8%)
Ampicillin	(AM, 10 µg)	108 (58.3%)	14 (7.5%)	63 (34.1%)
Amoxicillin+clavulanate	(AMC, 20+10 µg)	9 (4.8%)	28 (15.1%)	148 (80.0%)
Azithromycin	(AZM, 15 µg)	25 (13.5%)	18 (9.7%)	142 (76.7%)
Ceftazidime	(CAZ, 30 µg)	19 (10.2%)	32 (17.2%)	134 (72.4%)
Cefoxitin	(FOX, 30 µg)	21 (11.3%)	8 (4.3%)	156 (84.3%)
Cefotaxime	(CTX, 30 µg)	61 (32.9%)	8 (4.3%)	116 (62.7%)
Ciprofloxacin	(CIP, 5 µg)	63 (34.1%)	57 (30.8%)	65 (35.1%)
Chloramphenicol	(C, 30 µg)	74 (40.0%)	19 (10.2%)	92 (49.7%)

Gentamicin	(CN, 10 µg)	44 (23.7%)	4 (2.1%)	137 (74.1%)
Imipenem	(IPM, 10 µg)	4 (2.1%)	4 (2.1%)	177 (95.6%)
Nalidixic acid	(NA, 30 µg)	74 (40.0%)	19 (10.2%)	92 (49.7%)
Nitrofurantoin	(F, 300 µg)	43 (23.2%)	13 (7.0%)	129 (69.7%)
Tetracycline	(TE, 30 µg)	129 (69.7%)	2 (1.1%)	54 (29.1%)
Trimthoprim-sulfamethoxazole	(SXT,1.25/23.75 µg)	89 (48.1%)	6 (3.2%)	90 (48.6%)
Streptomycin	(S, 10 µg)	75 (40.5%)	19 (10.2%)	91 (49.1%)

Table 3. Multiple antibiotic resistance (MAR) index of *E. coli* isolates (n = 185).

No. of antibiotics	No. (%) of resistant *E. coli* isolates	MAR index
0	32 (17.2%)	0.00
1	18 (9.7%)	0.06
2	9 (4.8%)	0.12
3	13 (7.0%)	0.18
4	17 (9.1%)	0.25
5	28 (15.1%)	0.31
6	15 (8.1%)	0.37
7	11 (5.9%)	0.43
8	10 (5.4%)	0.50
9	14 (7.5%)	0.56
10	9 (4.8%)	0.62
11	6 (3.2%)	0.68
12	2 (1.1%)	0.75
13	0	0
14	0	0
15	0	0
16	1 (0.5%)	1.00

3. Discussion

3.1. E. coli Prevalence in Red Meat and Poultry

E. coli is recognized as one of the most important foodborne pathogens, with certain pathogenic strains capable of causing severe and potentially life-threatening illnesses in humans. Therefore, its presence in meat and meat products represents a significant concern for both food safety and public health. In the present study, *E. coli* was detected in 42% of poultry meat and poultry products and 38% of red meat and meat products, indicating a slightly higher prevalence in poultry.

The prevalence observed in red meat indicates a considerable level of contamination at the retail level and highlights its potential role as a vehicle for foodborne transmission. Similar prevalence rates have been reported by Elsawy et al. [19], who detected *E. coli* in 38.8% of red meat samples. In contrast, lower prevalence rates ranging from approximately 3% to 24% have been reported in other studies depending on sample type and processing conditions [20–22]. Conversely, Yi et al. [23] reported substantially higher contamination levels in beef samples (62.7%). These variations among studies may be associated with differences in slaughtering practices, hygienic conditions, processing procedures, sampling strategies, and geographical origin.

A similarly high level of contamination was observed in chicken meat and products in the present study. Previous studies have reported prevalence rates generally ranging from approximately 8% to 68% depending on poultry type and processing level [24–27]. The consistently high prevalence reported in poultry products suggests that poultry meat represents a favorable matrix for *E. coli* contamination and dissemination.

Overall, the present study demonstrated a higher prevalence of *E. coli* in poultry meat compared to red meat, which is consistent with several previous studies [24,28]. For instance, Lee et al. [28] reported that *E. coli* was more frequently isolated from poultry meat, with prevalence rates reaching 67.86% in chicken and 70.37% in ground turkey, compared to lower rates in pork chop (32.14%) and ground beef (20.00%). Similarly, Zhao et al. [24] found that 38.7% of chicken samples were positive for *E. coli*, whereas lower prevalence rates were observed in beef (19.0%), pork (16.3%), and turkey (11.9%) samples. In contrast to these findings, Yi et al. [23] reported a higher prevalence of *E. coli* in red meat compared to poultry, with contamination rates of 62.7% in beef and 53.2% in chicken. The higher prevalence observed in poultry meat may be attributed to multiple factors associated with poultry production and processing. Poultry generally harbors a higher intestinal bacterial load, increasing the likelihood of contamination during slaughter, particularly during evisceration. In addition, high-speed processing lines and extensive handling during poultry processing may facilitate cross-contamination. Furthermore, the structural characteristics of poultry skin provide a favorable surface for bacterial attachment and persistence. In contrast, contamination in red meat is often more surface-limited and may occur at lower levels under relatively more controlled processing conditions.

Among red meat samples, the highest contamination rate was observed in hamburgers (13/20, 65%), followed by minced beef (11/20, 55%) and meatballs (8/20, 40%), whereas diced beef and sausage showed considerably lower contamination levels (3/20, 15%). In poultry meat samples, chicken breast (15/20, 75%) and drumstick (13/20, 65%) exhibited the highest contamination rates, followed by chicken wing (11/20, 55%). Lower contamination levels were detected in chicken sausage (3/20, 15%), while *E. coli* was not detected in chicken burger samples**.**

A comparable distribution was reported by Elsawy et al. [19], who detected ***E. coli*** in 38.8% of red meat samples overall, with higher contamination rates in minced meat (44.4%) and launchow products (44.4%), followed by kofta (40.7%), sauces (37%), hamburger patties (33.3%), and sausage (33.3%). The agreement between these findings and the present study further supports the notion that processed and finely comminuted meat products represent higher-risk food categories due to increased handling and processing steps that facilitate microbial contamination and dissemination.

These findings demonstrate notable differences not only between meat types but also among product categories. The higher contamination rates observed in comminuted meat products, such as minced beef and hamburgers, may be attributed to increased surface area, disruption of muscle structure, and a greater risk of cross-contamination during processing. Similar findings were reported by Cagney et al. [7], who identified *E. coli* O157:H7 in minced beef and beef burger products. Grinding disrupts the natural protective barriers of whole-muscle meat and facilitates the distribution of microorganisms throughout the product, resulting in a more homogeneous contamination pattern, even when initial bacterial loads are low. In addition, repeated handling and contact with processing equipment may increase cross-contamination, reinforcing the role of minced and processed meat products as high-risk matrices for foodborne pathogens such as *E. coli*.

3.2. Virulence Gene Profiles of E. coli Isolates

The detection of virulence-associated genes in *E. coli* isolates recovered from retail meat products provides important insight into their pathogenic potential and public health significance. In the present study, *stx2* was the most prevalent gene (51.4%), followed by *eae* (37.3%), *hlyA* (13.0%), and *stx1* (6.5%). The predominance of *stx2* is particularly important because this gene is strongly associated with severe clinical outcomes such as hemorrhagic colitis and hemolytic uremic syndrome (HUS) [10]. Similar findings were reported by Zeinali et al. [27], reported that *stx2* (54.66%) was the most prevalent virulence gene in chicken meat isolates, followed by *stx1* (43.33%) and a lower frequency of *eae* (7.33%), indicating a similar dominance of *stx2* but a much lower presence of *eae* compared to the present findings.

The *eae* gene, which encodes the adhesion protein intimin, was detected in 37.3% of the isolates, indicating a considerable proportion of strains with enhanced colonization ability. This prevalence was higher than those reported in several previous studies, including Zeinali et al. [27] and Fatima et al. [26], who reported relatively low *eae* positivity (7-11%) rates in poultry-associated isolates. In contrast, Thierry et al. [29] reported a higher prevalence of *eae*-positive STEC isolates (47.6%) in animal-derived food sources. Such variations among studies may be associated with differences in animal source, geographical origin, hygienic practices, sampling strategies, and molecular detection methods.

The coexistence of *stx* and *eae* genes in the same isolates is of particular concern because these virulence determinants are commonly associated with enterohemorrhagic *E. coli* (EHEC) strains with enhanced pathogenic potential. In addition, in the present study, the *hlyA* gene, which encodes enterohemolysin, was detected in 13.0% of the isolates. Similar prevalence rates have been reported in retail meat isolates by Martínez-Vázquez et al. [30], although lower rates were observed by Thierry et al. [28]. Despite variations among studies, the presence of *hlyA* remains epidemiologically important because enterohemolysin may contribute synergistically with other virulence factors to increased disease severity.

Overall, the detection of *stx*, *eae*, and *hlyA* genes in *E. coli* isolates from retail meat products highlights the circulation of potentially pathogenic strains in the food chain. These findings further emphasize the importance of continuous monitoring of virulence-associated genes in foods of animal origin.

3.3. Antimicrobial Resistance

The emergence and dissemination of antimicrobial-resistant *Escherichia coli* in foods of animal origin represent a major public health concern worldwide because resistant strains may be transmitted to humans through the food chain. In the present study, high resistance rates were observed particularly for tetracycline (69.7%), ampicillin (58.3%), trimethoprim-sulfamethoxazole (48.1%), streptomycin (40.5%), nalidixic acid (40.0%), and chloramphenicol (40.0%). Similar resistance patterns have been reported in previous studies investigating meat-derived *E. coli* isolates [31–33]. The widespread resistance to these commonly used antimicrobials may be associated with their extensive use in food animal production, resulting in increased antimicrobial selection pressure.

The present study also demonstrated a high prevalence of multidrug-resistant (MDR) isolates (68.1%), which was higher than the MDR rates reported in several previous studies [31,32]. In addition, the MAR index ranged from 0.0 to 1.0, indicating the circulation of isolates with diverse resistance profiles. Notably, one isolate exhibited resistance to all tested antimicrobials, highlighting the potential public health risk associated with highly resistant *E. coli* strains in retail meat products.

Resistance to critically important antimicrobials such as ciprofloxacin was also considerable (34.1%) in the present study. Similar findings have been reported in retail meat isolates worldwide, although resistance levels vary depending on regional antimicrobial usage practices, farm management systems, and regulatory policies [23,32]. In Türkiye, Sahin et al. [34] reported the circulation of ESBL-producing and multidrug-resistant *E. coli* isolates in poultry, while Dishan et al. [35] demonstrated high levels of antimicrobial resistance and virulence-associated characteristics among STEC isolates recovered from retail chicken meat. These findings further support the role of poultry-associated food products as important reservoirs for antimicrobial resistance determinants and potentially pathogenic *E. coli* strains. The widespread occurrence of MDR *E. coli* in meat products further supports concerns regarding the role of retail foods as reservoirs for antimicrobial resistance determinants.

4. Materials and Methods

4.1. Sample Collection

A total of 200 samples, including 100 beef products (diced beef, minced beef, meatballs, hamburgers, and sausages) and 100 chicken products (chicken breast, chicken drumsticks, chicken wings, chicken sausages, and chicken burgers), were collected from various retail outlets and markets in Samsun, Türkiye, between November 2024 and February 2025. The samples were transported to the laboratory under cold-chain conditions and processed promptly upon arrival for analysis.

4.2. Isolation and Characterization of *E. coli*

The presence of *E. coli* in meat and meat products was determined using conventional culture-based methods. For analysis, 10 g of each food sample was homogenized with 90 mL of Maximum Recovery Diluent (Merck, Darmstadt, Germany) using a stomacher for 2 min. Subsequently, 0.1 mL aliquots from the homogenate were spread onto Violet Red Bile Agar (VRBA; Merck, Germany) and incubated at 37 °C for 24 h for coliform enumeration. Red–pink colonies on VRBA were transferred to Eosin Methylene Blue (EMB) agar (Merck, Germany) and incubated at 37 °C for 24 h. At least three presumptive colonies exhibiting a metallic sheen on EMB agar were subcultured on Tryptic Soy Agar (TSA; Merck, Germany) and incubated at 37 °C for 18–24 h for further biochemical confirmation. Presumptive *E. coli* isolates were preserved in cryovials containing Tryptic Soy Broth (TSB) supplemented with 20% glycerol and stored at −20 °C [36,37].

4.3. DNA Extraction

Genomic DNA from bacterial isolates was extracted using the boiling method. Isolates stored at −20 °C were first revived in Tryptic Soy Broth (TSB; Merck, Darmstadt, Germany) and incubated at 37 °C for 18–24 h. The cultures were then streaked onto Eosin Methylene Blue (EMB) agar, and colonies showing a metallic sheen were subcultured on Tryptic Soy Agar (TSA; Merck, Darmstadt, Germany) and incubated at 37 °C for 18–24 h. The obtained colonies were suspended in 200 µL of sterile deionized water in Eppendorf tubes. Denaturation was performed at 95 °C for 10 min using a dry block heater (Biosan TDB-120, Biosan, Lithuania). The samples were then centrifuged at 10,000 × g for 5 min at 4 °C (Hettich Universal 320R, Germany). The supernatant was transferred to a sterile Eppendorf tube and stored at −20 °C until molecular analysis [38].

4.4. PCR Confirmation of *E. coli* Isolates Using the *uspA* Gene

Presumptive *E. coli* colonies were confirmed by PCR targeting the *uspA* gene. All primers used in this study are listed in Table 4. The PCR mixture was prepared in a final volume of 25 µL containing 1× PCR buffer (500 mM KCl, 200 mM Tris–HCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.5 µM of each primer, 1 U Taq DNA polymerase, and 2 µL of template DNA. PCR reactions were performed using a thermal cycler (Bio-Rad MJ Mini, PTC-1148). The amplification conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 1.5 min, with a final extension at 72 °C for 5 min.

Table 4. Primers used in this study.

Primer	Primer Sequences (5′–3′)	Product size (bp)	Annealing (°C)	Reference
uspA-F	5′-CCGATACGCTGCCAATCAGT-3′	884	63	Chen & Griffiths [39]
uspA-R	5′-ACGCAGACCGTAGGCCAGAT-3′			
stx1-F	5′-AGTTAATGTGGTGGCGAAGG-3′	347	57	Fujioka et al. [40]
stx1-R	5′-CACCAGACAATGTAACCGC-3′			
stx2-F	5′-TTCGGTATCCTATTCCCGG-3′	589	57	Fujioka et al. [40]
stx2-R	5′-CGTCATCGTATACACAGGAG-3′			
eae-F	5′-GTGGCGAATACTGGCGAGACT-3′	890	55	Gannon et al. [41]
eae-R	5′-CCCCATTCTTTTTCACCGTCG-3′			
hlyA-F	5′-ACGATGTGGTTTATTCTGGA-3′	165	55	Fratamico et al. [42]
hlyA-R	5′-CTTCACGTGACCATACATAT-3′			

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4.5. Detection of Virulence Genes in E. coli Isolates by PCR

4.5.1. Detection of *stx1* and *stx2* Genes

The PCR mixture was prepared in a final volume of 25 µL containing 1× PCR buffer (500 mM KCl, 200 mM Tris–HCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.8 µM of each primer for *stx1* and *stx2*, 1 U Taq DNA polymerase, and 2 µL of template DNA. The amplification conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 45 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 7 min.

4.5.2. Detection of *hlyA* and *eae* Genes

The PCR mixture was prepared in a final volume of 25 µL containing 1× PCR buffer (500 mM KCl, 200 mM Tris–HCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.8 µM of each primer for *hlyA* and *eae*, 1 U Taq DNA polymerase, and 2 µL of template DNA. The amplification conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 7 min.

4.6. Agarose Gel Electrophoresis

PCR products were analyzed on 1.5% agarose gel prepared in 1× TBE buffer (Tris–borate–EDTA; 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) and stained with 0.5 µg/mL ethidium bromide. Electrophoresis was carried out at 80 V for 1 h using a horizontal electrophoresis system (multiSUB Horizontal System, Cleaver Scientific, UK). The *uspA* gene was visualized at 884 bp, *stx1* at 347 bp, *stx2* at 589 bp, *eae* at 890 bp, and *hlyA* at 165 bp under a UV transilluminator (Wise-UV WUV-L50, DAIHAN Scientific, Seoul, Korea).

4.7. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the *E. coli* isolates against various antibiotics was determined using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute [43,44]. The antibiotics used in this study are listed in Table 2. For this purpose, *E. coli* isolates were grown in Mueller–Hinton Broth (MHB; Merck, Darmstadt, Germany) at 35 °C for 18–24 h. After incubation, fresh cultures were adjusted to a turbidity equivalent to 0.5 McFarland standard (~1×10^8 CFU/mL) using a densitometer (DEN-1, Biosan, Riga, Latvia). Then, 100 µL of the bacterial suspension was uniformly spread on Mueller–Hinton Agar (MHA; Merck, Darmstadt, Germany) plates using a sterile cotton swab and allowed to dry for 15 min. Antibiotic discs were then placed on the agar surface, and the plates were incubated at 35 °C for 18–24 h. Following incubation, the diameters of inhibition zones around each disc were measured. The results were interpreted as susceptible, intermediate, or resistant according to the zone diameter breakpoints recommended by the Clinical and Laboratory Standards Institute [45].

4.8. Multidrug Resistance (MDR) and Multiple Antibiotic Resistance (MAR) Index

Isolates showing resistance to at least one agent in three or more antimicrobial classes were classified as multidrug-resistant (MDR) according to the criteria proposed by Magiorakos et al. [46]. The multiple antibiotic resistance (MAR) index for each isolate was calculated using the following formula: MAR index=a/b where *a* represents the number of antibiotics to which the isolate was resistant and *b* represents the total number of antibiotics tested [47]. High MAR index values were considered indicative of exposure to environments with frequent antimicrobial use.

5. Conclusions

In conclusion, the present study demonstrated that retail beef and chicken meat products may serve as important reservoirs of potentially pathogenic and multidrug-resistant *E. coli*. The coexistence of virulence-associated genes and antimicrobial resistance determinants among the isolates increases the potential public health risk associated with contaminated meat products. Therefore, continuous surveillance programs, prudent antimicrobial use in food animal production, and improved hygienic practices during slaughtering and meat processing remain essential for limiting the dissemination of antimicrobial-resistant *E. coli* throughout the food chain. Furthermore, additional molecular characterization of resistance and virulence determinants, particularly through whole-genome sequencing-based studies, may provide deeper insight into the epidemiology and transmission dynamics of pathogenic *E. coli* strains associated with retail meat products.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
CFU	Colony-Forming Unit
CLSI C	Clinical And Laboratory Standards Institute
EHEC	Enterohemorrhagic *Escherichia Coli*
EMB	Eosin Methylene Blue Agar
HUS	Hemolytic Uremic Syndrome
MDR	Multidrug-Resistant
MAR	Multiple Antibiotic Resistance
MHA	Mueller–Hinton Agar
MHB	Mueller–Hinton Broth
STEC	Shiga Toxin-Producing Escherichia Coli
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
VRBA	Violet Red Bile Agar

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