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Characterization of multiple resistance genes of *Escherichia coli* strains isolated from food and clinical samples

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ABSTRACT

Multi-drug resistance (MDR), which is formed by the development of antimicrobial resistance, which is one of the defense mechanisms in bacteria, has pushed human beings to constantly seek new antimicrobial agents in the fight against these microorganisms. Speed and precision are very important in identifying resistance in strains with MDR that reach humans in an open system through the consumption of contaminated food or water. The main aim of this study was the molecular characterization of ESBL gene variants (bla_{TEM}, bla_{OXA}, bla_{SHV}, and bla_{CTX-M}), integron genes (*int1, int2,* and *int3*), and sulphonamide (*sul1, sul2,* and *sul3*) resistance genes by PCR from *E. coli* isolates originated from food and clinical samples. A total of 17 sets of primers were used for phylogenetic identification, molecular detection, and resistance and integron gene characterization of phenotypically identified *E. coli* isolates. The 45 red meat samples were collected from local markets, which are located in four (Adıyaman, Gaziantep, Kahramanmaraş, and Hatay) different provinces, clinical isolates were obtained from urine samples of patients with UTI from Şanlıurfa Mehmet Akif İnan Training and Research Hospital. Three gene groups were screened with 3 multiplex PCR applications in 63 food and 33 clinical isolates found to be *E. coli* by molecular identification. In terms of the 3 gene groups screened in food samples, the highest rates were found in the bla_{SHV} gene at 44.44%, the *sul1* gene at 69.84% and the *int2* gene at 73.02%; in clinical samples, it is listed as bla_{CTX-M} gene at 15.15%, *sul2* gene at 81.82% and *int1* gene at 54.55%. In terms of 3 gene groups scanned, the presence of 3 or more genes, including at least one gene from each gene group, was detected in 31 isolates from food samples and 2 isolates from clinical samples. Overall, it can be said that the high frequency of MDR genes isolated from *E. coli*-contaminated red meat samples and clinical samples gives a clue about the overuse of antibiotics in Türkiye.

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Introduction

The significant increase in the incidence of microbial infections triggered the intensive use of antimicrobial agents for treatment and caused the development of resistance mechanisms against these agents in bacteria (1). Therefore, the reason for the development of resistant bacterial species/strains is the pressure exerted by antibacterial agents (2, 3). The emergence and rapid spread of new resistances has caused the response to standard treatment to be ineffective and have made antimicrobial resistance a global public health problem (1).

Bacterial antibiotic resistance, which started to be observable with the use of antibiotics in the fight against pathogenic bacteria in the 1940s followed a course in parallel with the increase in antibiotics, causing resistance mechanisms to become more complex and resistant to more than one agent (4).

Multiple drug resistance (MDR) is an acquired resistance of microorganisms to drugs that are characterized by different chemical structures and different mechanisms of action. The cost of treatments and the difficulty of the process increases the threat to public health, and studies have shown that resistance to a single agent is in compatible correlation with resistance to more than one agent in bacterial populations (5). In order to combat infections caused by multi-drug resistant pathogens, it is necessary to know the cause of the resistance and to develop control strategies accordingly (5). This is based on the characterization of multidrug-resistant pathogens. Although traditional phenotypic methods such as disc diffusion and microdilution are used to determine antimicrobial resistance, these methods are insufficient to determine the mechanism of resistance at the molecular levels. Molecular techniques such as Polymerase Chain Reaction (PCR) and DNA sequencing have made it possible to identify multiple resistance genes or gene communities and to monitor resistance-determining markers, thus closing the gap between the traditional methods (6).

This study, presents information about the characterization of multi-resistant *E. coli* isolates isolated from food and clinical samples by PCR.

Materials and Methods

Food and clinical samples

In this study, genotypic identification of phenotypically identified *E. coli* strains isolated from food and clinical samples and characterization of extra spectrum β-lactamases ESBL gene variants (sulphonamide resis-

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tance genes and integron genes) in these strains by PCR were performed. The meat samples used in this study were obtained from local markets located in Adıyaman, Gaziantep, Hatay, and Kahramanmaraş provinces from May to August 2019. Of the collected meat samples, 25 of them were beef, 10 of them were lamb, and 10 of them were goat meat. The clinical samples were obtained from Şanlıurfa Mehmet Akif İnan Training and Research Hospital in broth from patients with urinary tract infection (UTI).

Isolation and detection of *E. coli*

For the isolation of bacteria, 25 g food samples weighed were homogenized in suspension salt 1× phosphate-buffered saline (PBS) (225 ml) with a pH of 7.6. Bacteria samples taken from homogenates were inoculated on MacConkey and Eosin methylene blue (EMB) agar media and colony-forming unit (CFU) numbers were determined. Greenish-turquoise-colored colonies were observed on MacConkey medium and red to purple colonies on EMB agar medium were detected. Samples taken from colonies were serially diluted after 18 hours of incubation at 37°C. 1 ml of the diluted samples with 1×10^6 CFU/ml were taken and boiled in a hot water bath at 95°C for 5 minutes. Samples were taken after boiling and centrifuged at 17,000 g for 5 minutes. The supernatants containing isolated DNA were taken and kept at -20°C until use (7, 8).

Clinical samples were taken from the hospital in broth and incubated at 37°C for 18 hours. After the incubation, 1 ml was taken from the samples diluted to 1×10^6 CFU/ml and boiled at 95°C for 5 minutes. Then, centrifuged at 2,000 rpm for 5 minutes. The supernatants were collected by centrifugation and stored at -20°C to be used as DNA templates for PCR (8).

Molecular identification of *E. coli* isolates

Escherichia coli strains phenotypically detected from isolates of food and clinical origin were subjected to PCR to confirm them at the molecular level. The PCR method was performed by amplifying the *uspA* gene, one of the universal stress proteins (usp) genes that express five small Usp proteins in *E. coli*. For validation the PCR was first applied to the DNA template isolated from a reference microorganism (*E. coli* ATCC 25922). The same PCR procedure was then used on testing all isolates of food and clinical origin. The PCR was performed as described by Chen & Griffiths (9) (Table 1).

Phylogenetic characterization of *E. coli*

Phylogenetic identification of *E. coli* isolates was performed by PCR using 6 primer pairs (chuA, yjaA, arpA1, arpA2, trpA, and TSPE4C2) as described by Clermont (10). *E. coli* isolates were classified into 8 different phylogroups as A, B1, B2, C, D, E, F, and clade 1. The identification process took place in 3 steps. The first step was performed using four primer pairs (chuA, yjaA, TSPE4C2 and arpA1) as quadruplex PCR and identification was made based on the genes that were amplified (11). The second step was PCR using the arpA2 primer, the presence of a 301 bp band indicated the inclusion of the sample in class E, and the presence of the amplicon of 219 bp indicated the inclusion of the sample in one of the two classes (A or C) (Table 1). Multilocus sequence typing (MLST) screening is recommended for specimens with positive phenotypic identification but negative phylogenetic classification (12).

Identification of ESBL gene variations in *E. coli* isolates

Four primer pairs were used to identify ESBL gene

Table 1. *E. coli* genotypic and phylogenetic expression genes and primer sequences used for PCR amplification.

Purpose of Use	Gene	Primers (5'→3')	Amplicon length (bp)	Reference
Genotypic confirmation (*E. coli*-specific)	*uspA*	F: CCGATACGCTGCCAATCAGT R: ACGCAGACCGTAGGCCAGAT	884	(9)
Phylogenetic identification	*chuA*	F: ATGGTACCGGACGAACCAAC R: TGCCGCCAGTACCAAAGACA	288	(10)
	yjaA	F: CAAACGTGAAGTGTCAGGAG R: AATGCGTTCCTCAACCTGTG	211	
	Tspe4.C2	F: CACTATTCGTAAGGTCATCC R: AGTTTATCGCTGCGGGTCGC	152	
	arpA1	F: -AACGCTATTCGCCAGCTTGC R: TCTCCCCATACCGTACGCTA	400	
	arpA2	F: GATTCCATCTTGTCAAAATATGCC R: GAAAAGAAAAAGAATTCCCAAGAG	301	
	trpA	F: AGTTTTATGCCCAGTGCGAG R: TCTGCGCCGGTCACGCCC	219	

Table 2. *E. coli* ESBL gene variants and primer sequences used for PCR amplification.

ESBL Gene Variant	Primers (5'→3')	Amplicon Length (bp)	Reference
bla_{CTXM}	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	551	(13)
bla_{OXA}	F: TCAACTTTCAAGATCGCA R: GTGTGTTTAGAATGGTGA	610	
bla_{SHV}	F: TTATTTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCCGG	727	
bla_{TEM}	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	1080	

variants in isolates, selecting bla_{CTXM}, bla_{OXA}, bla_{SHV}, and bla_{TEM} genes as target genes. Identification was performed by multiplex PCR method in which four genes were screened simultaneously. A multiplex PCR was performed as described previously (13) (Table 2).

Identification of sulphonamide and integron resistance genes in *E. coli* isolates

sul1, *sul2*, and *sul3* genes, which are sulphonamide resistance genes, were identified by a multiplex PCR method using the *E. coli* isolates. Amplicon lengths of the amplified regions are given in Table 3.

The identification of different integron classes in isolates was made by a multiplex PCR containing the determination of three integron genes, *int1*, *int2* and *int3* (14) (Table 4).

Results

Genotypic identification of *E. coli* from food and clinical isolates

In this study, a total of 45 red meat samples (25 bovine and 20 ovine) were obtained from local markets. The main aim of this study was to detect and determine *E. coli* that was found in the red meat samples. For genotypic identification, the presence of the *uspA* gene, which is specific for *E. coli*, was detected by PCR for confirmation of the desired bacteria. In total, it was determined that 67 colonies phenotypically belonged to *E. coli* as color differences suggested after incubation on chromogenic media (MacConkey agar, EMB). As a result of PCR-based genotypic screening for the presence of the *uspA* gene in the phenotypically detected colonies, and it was determined that only 4 colonies did not belong to *E. coli* and the other 63 colonies were confirmed as *E. coli*. It was observed that the phenotypic description was compatible with the Genotypic description with a rate of 94%. All colonies that were detected from ovine samples were *uspA* positive, while in bovine samples the rate of positivity was 90.2%.

Clinical isolates, which were phenotypically positive for *E. coli* after the chromogenic tests, were confirmed by PCR and all of the 33 isolates were found to be *uspA* positive.

Phylogenetic grouping

In food samples, quadruplex PCR was used for phylogenetic classification and it was determined that the samples were belonged to different phylogroups. Of the 63 *uspA* positive isolates, it was determined that 34.9%, 14.3%, 11.1%, 7.9%, 3.2%, 3.2%, and 4.8% of the samples belonged to group A, B1, B2, C, D, E, and F, respectively. However, 20.6% of the samples did not belong to any of these groups and classified as group U (unidentified), which required a more detailed examination using MLST.

Identification of ESBL gene variants in *E. coli* isolates

None of the four studied *bla* genes were found in 16 (25.4%) isolates. The bla_{CTX-M} gene was found in 8 isolates (12.7%) and 7 of them were from bovine isolates and 1 from ovine samples. Fourteen isolates (22.2%) were positive for the bla_{OXA} gene, of which 8 (21.6% of bovine isolates) were bovine and 6 (23.1% of ovine isolates) were ovine meat isolates. The bla_{SHV} gene was found in 28 isolates (44.4% of meat isolates), including 16 ovine isolates (61.53% of ovine isolates) and 12 bovines (32.43% of bovine isolates) isolates. The bla_{TEM} gene was found in 3 ovines (11.54% of ovine isolates) and 6 bovine (16.22% of bovine isolates) isolates. All of the 4 *bla* genes were found together in only one of the 63 isolates (1.6%) and 3 genes also were found together in only one isolate (1.6%). Two of the four genes were found together in 7 isolates (11.1%) whereas a single gene was found in 38 isolates (60.38%) as presented in Table 5. In clinical isolates, only 5 out of 33 samples were positive in terms of *bla* genes, and all of them had only the bla_{CTX-M} gene (Table 6).

Sulphonamide resistance genes in *E. coli* isolates

Detection of three of the sulphonamide resistance genes (*sul1*, *sul2*, and *sul3*) was included to the current study. Of all of the 63 food-borne *E. coli* isolates, 13 (4 ovines and 9 bovines) of them did not have any of the three *sul* genes. Two of the *sul* resistance genes were found in 11 of the isolates (17.46%) and all three genes were found in only two isolates (3.17%). In 38 (60.3%) isolates, only one of the *sul* genes was detected (Table 5).

As can be seen in Table 6, of the 33 clinical isolates, *sul1* gene was positive in 39.4% (13), *sul2* in 81.8% (27),

Table 3. *E. coli* sulphonamide resistance genes and primer sequences used for PCR amplification.

Sulphonamide Resistance Gene	Primers (5'→3')	Amplicon Length (bp)	Reference
sul 1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433	(34)
sul 2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	293	
sul 3	F: GAGCAAGATTTTTGGAATCG R: CTAACCTAGGGCTTTGGATAT	750	

Table 4. *E. coli* integron genes and primer sequences used for PCR amplification.

Integron Gene	Primers (5'→3')	Amplicon Length (bp)	Reference
int1	F: CCCGAGGCATAGACTGTA R: CAGTGGACATAAGCCTGTTC	160	(14)
int2	F: CACGGATATGCGACAAAAAG R: GATGACAACGAGTGACGAAA	788	
int3	F: GCCTCCGGCAGCGACTTTCAG R: ACGGATCTGCCAAACCTGACT	979	

Table 5. PCR results of resistance genes and their variants in *E. coli* isolated from meat samples.

Sample #	bla_{CTX-M}	bla_{OXA}	bla_{SHV}	bla_{TEM}	*sul 1*	*sul 2*	*sul 3*	*int 1*	*int 2*	*int 3*
1	+	+								
2						+		+		
3					+			+	+	
4								+		
5			+		+			+	+	
6			+		+			+		
7					+	+		+	+	
8		+			+		+	+	+	
9		+			+		+	+	+	
10			+		+	+		+	+	
11			+		+		+	+	+	
12		+			+	+		+	+	+
13			+		+					
14			+		+				+	
15			+		+			+	+	
16			+		+		+	+	+	
17			+		+			+	+	+
18	+	+	+	+	+			+	+	
19			+		+			+	+	
20				+	+		+		+	
21			+						+	
22			+						+	+
23				+				+		
24			+		+					
25			+		+					
26		+							+	
27			+				+		+	
28			+		+				+	
29		+	+	+	+				+	
30			+		+				+	
31		+			+			+	+	
32		+						+	+	+
33			+		+					
34								+		
35		+							+	
36		+	+		+			+		+
37				+	+			+		
38	+					+			+	
39			+						+	
40	+					+			+	
41	+		+		+		+	+		
42									+	
43	+		+		+		+	+	+	
44					+	+	+	+	+	
45					+	+				
46					+	+		+	+	
47	+					+		+	+	
48						+		+	+	
49						+	+	+	+	
50					+			+	+	
51								+	+	+
52					+			+	+	
53					+		+	+	+	

Sample #	bla_{CTX-M}	bla_{OXA}	bla_{SHV}	bla_{TEM}	*sul 1*	*sul 2*	*sul 3*	*int 1*	*int 2*	*int 3*
54				+				+	+	
55	+	+						+	+	
56				+	+			+	+	
57					+			+	+	
58					+			+		
59					+			+		
60				+			+	+	+	
61				+	+			+	+	
62					+			+	+	+
63			+		+			+	+	
64			+					+	+	
65		+			+			+	+	
66							+			
67						+				
68		+		+	+					
69			+		+					
70	+		+							
71			+		+					
72			+		+			+	+	

Table 6. PCR results of resistance genes and their variants in clinical *E. coli* isolates.

Sample #	bla_{CTX-M}	bla_{OXA}	bla_{SHV}	bla_{TEM}	*sul 1*	*sul 2*	*sul 3*	*int 1*	*int 2*	*int 3*
1						+				
2						+				
3					+					
4						+	+	+		
5						+	+	+		
6						+		+		
7	+				+		+	+		
8					+	+	+	+		
9					+	+	+	+		
10					+	+	+	+		
11	+				+	+				
12					+		+	+		
13					+	+				
14					+	+				
15						+				
16	+					+				
17						+				
18						+				
19						+	+			
20	+					+				
21						+		+		
22						+	+	+		
23						+	+	+		
24						+		+		
25					+		+	+		
26						+	+	+		
27						+	+	+		
28					+		+	+		
29						+	+			
30					+	+				
31						+		+		
32	+				+		+	+		
33						+				

sul3 in 48.5% (16). It was determined that all three genes were found together in 9.1% (3) of the isolates, *sul1* and *sul2* in 12.1% (4), *sul1* and *sul3* in 15.2% (5), and *sul2* and *sul3* genes in 24.2% (8).

Integron genes in *E. coli* isolates

In the current study, in which three *int* genes (*int1, int2*, and *int3*) were screened by PCR. Of the 63 isolates isolated from meat samples, none of these genes were found in 17.5% of the isolates (11 isolates). *int1* and *int2* in 44.4% (28 isolates, 11 ovines and 17 bovines), *int1* and *int3* in 1.6% (1 isolate), *int2* and *int3* in 1.6% (1 isolate), and all three genes were positive in 7.94% (5 isolates) of them. Two of the isolates with triple resistance genes originated from ovine and three of them from bovine samples. A single *int* gene was found in 27% (17) of the isolates. The highest rate of occurrence among these three genes was found to be *int2* with 73.1% (46), followed by *int1* (63.5%, 40 isolates), and *int3* (11.1%, 7 isolates) genes.

Among the *int* genes investigated in clinical samples, the *int1* gene was found in 54.6% (18) of the 33 isolates, while *int2* and *int3* genes were not found in any of the isolates.

Co-existence of β-lactam and sulphonamide resistance genes

The rate of having at least one *bla* gene and the *sul1* together is 42.3% (11) in ovine samples, while it is 37.8% (14) in bovine samples. The rate of having at least one *bla* gene and *sul2* together was 5.4% (2) in bovine, and this association was not observed in ovine samples. As a result of the combination of at least one *bla* gene and *sul3*, it is again 5.4% (2) only in bovine samples. At least one *bla* + *sul1* + *sul2* combination was observed only in ovine samples with a rate of 7.7%. At least one combination of *bla* + *sul1* + *sul3* is found of 19.2% in ovine samples and 2.7% of bovine samples. At least one *bla* + *sul2* + *sul3* combination was found with a rate of 2.70% only in bovine samples, and no sample with all of the three *sul* genes together with *bla* gene/genes was found together.

Co-existence of β-lactam and integron genes

When the food samples are evaluated in terms of having at least one *bla* gene and integron gene/genes together; the combination of 1 ≥ *bla* + *int1* was found in 4.4% (1) of ovine samples and 5.4% (2) in bovine samples. The combination of 1 ≥ *bla* + *int2* was found in 15.4% (4) ovine samples and 18.9% (7) in bovine samples. The combination of 1 ≥ *bla* + *int3* was not found in any of the isolates. 1 ≥ *bla* + *int1* + *int2* combination was observed in 38.5% (10) ovine and 27.1% (10) bovine samples. 1 ≥ *bla* + *int1* + *int3* combination with a rate of 2.7% (1) only in bovine samples and 1 ≥ *bla* + *int2* + *int3* combination was observed only in ovine samples with a rate of 3.9% (1). The combination of 1 ≥ *bla* + *int1* + *int2* + *int3* was observed with 7.7% (2) in ovine samples and 2.7% (1) in bovine sample.

While *bla* + *int1* combination was found in only 2 (6.1%) of the clinical samples, other combinations were not observed in the studied 33 clinical samples.

Co-existence of sulphonamide and integron genes

The rate of having at least one *sul*+*int1* gene together in meat samples was found to be 3.9% (1) in ovine and 8.1% (3) in bovine samples. The 1 ≥ *sul* + *int2* gene combinations investigated in the study were found in 7.7% (2) of ovine and 16.2% (6) of bovine samples. While 1 ≥ *sul* + *int3* combination and 1 ≥ *sul* + *int2* + *int3* combination was not found in any sample; 1 ≥ *sul* + *int1* + *int2* combination was found in 46.2% (12) ovine and 32.4% (12) bovine samples. The combination of 1 ≥ *sul* + *int1* + *int3* was observed only in bovine samples with a rate of 2.7% (1). The combination of 1 ≥ *sul* + *int1* + *int2* + *int3* was found only in ovine samples and its ratio to all ovine samples was 11.5% (3).

In clinical samples, 1 ≥ *sul* + *int1* combination was found at a rate of 54.6% (18). When we evaluate the individual combinations of *sul* genes with *int1* with a ratio of 12.1 (4), 15.6% (5) and 18.2% (6) for *sul2* + *int1*, *sul1* + *sul3* + *int1*, and *sul2* + *sul3* + *int1* combinations, respectively. It was found that 3 (9.1%) of the 33 isolates did not contain any of the *sul* and *int* genes together.

Co-existence of β-lactam, sulphonamide, and integron genes

The co-occurrence rates of the ESBL gene variants, sulphonamide resistance and integron genes were checked for each sample (both meat and clinical), 1 ≥ *bla* + 1 ≥ *sul* + 1 ≥ *int* gene association for food samples was found in 31 isolates, in 15 ovine samples and 16 bovine samples and it was observed only in 2 clinical isolates.

Discussion

Resistance is defined as a microbe's insensitivity to an antimicrobial drug when compared to other isolates of the same species. Despite the commercialization of several new drugs, the development of resistance among infectious microorganisms is increasing, particularly in patients with prolonged drug exposure (15). Since their discovery, MDR-producing strains have been continuously isolated throughout the world, with an increase in their occurrence among clinical strains, isolates from healthy humans, and foods derived from animals (16). Furthermore, these bacteria have been found to be widespread in the environment, food, and clinical samples. However, there are few reports in Türkiye on the persistence of antibiotic resistance in pathogenic bacteria and the molecular basis for resistance phenotype (17).

As a result of the research, which was done by Uyanık in 2022, ESBL-producing *E. coli* isolates from ready-to-consumption food samples were screened and 87 colonies were suspected as *E. coli* in the chromogenic medium and studied for the presence of *uspA* gene by PCR and all were positive (18). The current study also presented a similar rate (90.2%) in the confirmation of *E. coli* by PCR. In the study of Uyanık in 2022, ESBL-producing bacteria in prepared sandwich samples in hospital canteens were screened and the presence of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes on 32 *E. coli* bacterial isolates was observed (18). Montso et al. (2019) examined the presence of ESBL in cattle feces and raw beef by ERIC PCR, and 85.5% of the *E. coli* isolates were found to have bla_{TEM}, 69.6% bla_{SHV}, and 58% $bla_{CTX\text{-}M}$ genes (19). In another study, ESBL-producing *E. coli* was isolated and identified in slaughtered cattle and sheep stool samples in Portugal in 2013, it was reported that the $bla_{CTX\text{-}M}$ gene was found in 9.3% of cattle samples and 5.5% of sheep samples. In addition, the com-

bination of the bla_{TEM} gene and bla_{CTX-M} gene, which is another β-lactamase gene screened in the study, was found only in sheep samples. This study's results are consistent with our study data, with both the presence of the bla_{CTX-M} gene and the observation of the bla_{TEM} + bla_{CTX-M} combination in both animal groups (20). ESBL-producing *E. coli* was detected by culturing methods in 17 of 101 isolates from sheep meat sold at local markets in the city of Zagazig, Egypt. It was stated that all of them were confirmed as *E. coli* by PCR. When the bla genes were screened on these isolates, it was reported that the bla_{CTX-M} gene was found at the highest rate (in 7 isolates), followed by bla_{TEM} with 3 isolates, and bla_{SHV} with 2 isolates (21).

The presence of the four *bla* genes tested in the present study in clinical isolates of ESBL-producing *E. coli* was also studied Abrar et al. 2019 using single PCR and the highest rate was observed in the bla_{CTX-M} gene with a rate of 72% (22). This study results are in line with the data of the current study. Kluytmans et al. (2013) reported the presence of bla_{CTX-M} in 34, bla_{TEM} in 9, and bla_{SHV} in 1 of 44 ESBL-producing *E. coli* isolates obtained from humans (23). Similarly, another study stated that the bla_{CTX-M} gene was found in 79.31% of isolates from patients with UTI, followed by bla_{TEM} and bla_{OXA} in 58.62%, while bla_{SHV} was not observed in any of the samples (24).

Li et al. screened all three *sul* genes by PCR in 166 *E. coli* isolated from chicken and pork meat, the *sul1* gene was observed in 16.9%, *sul2* 32.5%, and *sul3* 13.9% of the isolates (25). Similarly, Badi et al. studied *sul* genes on *E. coli* isolated from animals and animal products and the presence of *sul1* gene was observed in only 1, *sul2* gene in 4, and *sul3* gene in 3 of 65 isolates (26). Lerma et al. screened 3 of the sulphonamide resistance genes to detect the presence of antimicrobial resistance genes (AMRs) on *E. coli* isolated from slaughterhouse surfaces and meat (goat and sheep) samples (27). The *sul3* gene was found with the highest rate on 25 (78%) of the slaughterhouse surfaces, followed by the *sul2* + *sul1* combination. In addition, the *sul3* gene was detected in all meat samples in the study.

Arabi et al. obtained 144 *E. coli* isolates from clinical samples in Iranian hospitals and 116 of them were found to have *sul1*, 96 with *sul2*, and 3 with *sul3* gene (28). In the same study, 122 isolates of bla_{TEM} genes, 76 isolates of bla_{SHV} genes, and 37 isolates of bla_{CTX-M} genes were found in terms of β-lactam genes. In addition, 68 isolates were positive for *int1* from integron genes (28). In the study conducted to determine the sulphonamide resistance genes in *E. coli* isolates that cause UTI in Uruguayan children, resistance to trimethoprim/sulfamethoxazole was detected in 37.9% of the samples, and it was stated that *sul* resistance gene was found in 85.5% of them (29).

As a result of gene characterization of 11 cephalosporin-resistant *E. coli* samples isolated from various meat samples, including 27 cattle and 1 sheep, obtained from fecal samples from animal farms and ready-to-eat foods in Tunisia, 3' bla_{CTX-M}+ *sul1* gene combination was found in 3 of them and it was reported that the same samples were positive for 1st class of integron gene (30). In another study, the presence of *int1* and *int2* genes in hospital-acquired *E. coli* isolates was screened, and *int1* and *int2* genes were detected in 47.05% of the isolates with multiple resistance (31). The *int1* gene was found in 97% of 33 MDR *E. coli* isolates isolated from patients hospitalized in Iran, and it was stated that there were similarities in terms of integron gene cassettes in the samples. In the same study, the presence of the *int1* gene was reported in 87.5% (7/8) of the ESBL-positive isolates (32).

Resistance development, which is a strong defense mechanism for bacteria, increases its threat to human beings' day by day. Having multiple resistance mechanisms reinforces this threat. Cause the threat Bacterial diversity with MDR continues its upward trend accelerating through MGE via horizontal gene transfer. *E. coli* strains encountered among bacteria with this tendency are frequently isolated from samples, although the source is variable (food and clinical origin). In this context, gene screening was performed by PCR application for the detection of multiple resistance genes on *E. coli* isolates isolated from different samples of food and clinical origin.

Four ESBL gene variants, 3 sulphonamide resistance genes, and 3 genes in terms of integrons thought by the scientific world to take part in the transfer of resistance genes were screened in isolates with 3 different multiplex PCR applications. The study differs from the literature in that the 3 gene groups were screened as multiplex under specific conditions, and the D group *OXA* gene was screened under the same conditions as the A group genes found in studies in the literature in terms of *bla* genes. The difference is not only limited to the PCR conditions of the genes screened together. It is thought that the contribution will provide to the literature is important in terms of giving double and triple positive correlations of the 3 groups of data scanned in the current study, and the triple correlation relationship detail is important. In addition, this study is also important in that it allows a comparison of the possible MDR risk in terms of resources by comparing the data obtained from two main sources. The study importance increases in terms of evaluating resistance gene connections with integrons, which are frequently mentioned in resistance gene transfers of two different resistance groups.

Escherichia coli, which are natural members of the gastrointestinal systems of humans and animals, are among the important virulent factors that reach humans through plants and animal-related foods due to their rapid spread in open systems (33). Uropathogenic *Escherichia coli* (UPEC), which are clinically derived from *E. coli* isolates found in many environments including hospital-acquired environments. They play the role of the primary causative factor of UTIs, which is one of the common nosocomial and community-acquired infections. Empirical antibiotic applications in the treatment of infection have triggered the development of MDR in pathogenic and commensal *E. coli*, including UPECs (24).

With multiplex PCR, it is possible to scan food and clinical samples in a short time by creating molecular-based kits in which the gene groups used in the study will be applied together, and the number of investigated genes can be increased. Thus, food safety detection will be accelerated, and the spread of resistance and health expenditures can be reduced by using antimicrobials suitable for the pathogen of clinical origin. In the present study, both in food and clinical samples, the number of isolates with genes from all three groups was at a substantial level, and this level was higher in foods. For this reason, it is important to inform producers about the threat of MDR spread about the importance of hygienic conditions for the preparation, storage and consumption of food source animals

and animal products in order to prevent the spread of food-borne MDR pathogenicity and to remove it from being a virulent factor for humans.

Future studies will be valuable to screen the response of bacteria to antibiotics phenotypically in order to understand whether the resistance genes obtained in PCR screening of the molecular basis underlying MDRs show resistance functionally or not.

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Conflict of interests
Authors have no conflict of interest to declare.

Author's contribution
Concept: Birgül YELİMLİBAĞ and Muhsin AYDIN; Sample Collection: Birgül YELİMLİBAĞ and Muhsin AYDIN; Methodology and applications: Birgül YELİMLİBAĞ and Muhsin AYDIN; Literature review, data collection or processing: Birgül YELİMLİBAĞ and

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