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Research Article

INVESTIGATION OF ESβL, AMP-C, CARBAPENEMASE PRODUCING *ESCHERICHIA COLI* STRAINS ANTIMICROBIAL RESISTANCE PROPERTIES ISOLATED FROM RED MEAT IN RETAIL BY MIC, PCR, AND LAMP PCR ANALYSIS

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ABSTRACT. In this study; phenotypic antibiotic resistance properties of ESβL, Amp-C, and carbapenemase producing *Escherichia coli* strains isolated from red meats offered for retail sale were analyzed by E-Test and disc diffusion test. Antibiotic resistance genes were determined by PCR and LAMP PCR methods. Twelve *E. coli* strains were isolated from 120 red meat samples that were collected and it was verified by a rapid identification system (BBL Crystal). In 10 isolates, as a result of the disk diffusion test of the isolates, ESβL activity was determined; resistance was seen to ampicillin and tetracycline with 91.6%, cefotaxime with 83.33%, chloramphenicol, ciprofloxacin and nalidixic acid with 75%, azithromycin, and sulfamethoxazole/trimethoprim with 66%, gentamicin, and ceftazidime with 41.6%, and tigecycline with 33.3%; no resistance to meropenem was observed. In addition, all isolates were resistant to cefoxitin. With the E-test, ESβL activity was found in 1 isolate; Amp-C and carbapenemase activity were not detected in any of the isolates. As a result of PCR, when examined in terms of ESβL activity, in 4 isolates, the *CTX-M1* gene was detected, and the *SHV* gene was determined in all *E. coli* isolates; Amp-C and carbapenemase genes were not detected. When carbapenemase genes were examined by LAMP PCR, two isolates were positive and all of the 6 carbapenemase genes that were examined were found in these two positive isolates. Our study, it was aimed to determine the resistance genes of red meats within 24 hours of pre-enrichment without agent isolation, and the same results were obtained, in addition. Detection of these resistance genes in retail red meat poses a risk to public health. In line with the concept of one health, it is important for public health to detect the resistance genes detected in red meat and take the necessary measures to reduce this resistance.

Keywords: *Escherichia coli, PCR, ESβL, Amp-C, carbapenemase.*

INTRODUCTION

Antibiotic resistance has become threatening to human health since human health is affected by animal health [1]. β-lactam antibiotics are the most widely used antibiotics in both humans and animals. Antimicrobial resistance (AMR), especially resistance to several antibiotics (Multidrug Resistance, MDR), has been one of the biggest threats to global public health [2]. Due to the uncontrolled use of antibiotics in animals, it is emphasized that strains resistant to antibiotics such as Extended-Spectrum Beta-lactamases (ESβL) and Ampicillin-C (Amp-C) resistance can spread through the food

chain, contact, or water sources [3]. It has been reported that genetic elements encoding resistance in ESβL and Amp-C-producing *Enterobacteriaceae* are transported between the same and/or different bacteria species, and thus they rapidly spread throughout the world [4]. The most researched genes related to resistance in animals are bla*CTX-M-1*, bla*CTX-M-2*, bla*CTX-M-14*, bla*CTX-M-15*, bla*TEM-52,* and bla*SHV-12* [5]. Bacteria that produce zoonotic ESβL with the *CTX-M* gene, it decreases treatment options causing a direct impact on human health. [6]. An interdisciplinary approach is required with the One Health concept to control ESβL-produced *Escherichia coli (E. coli)* in farm animals [7]. Plasmid-originated Amp-C type β-lactamases appear together, especially with ESβLs [8, 9]. Carbapenemases that hydrolyze carbapenems continue to increase in recent years [10]; it is being isolated from many animals [11, 12]. When the distribution of ESβLs is considered; within Europe, the *CTX-M-1* group (*CTX-M-1* and *-15*) is common. *CTX-M-9* group (*CTX-M-9* and *-14*) was mostly detected in animals from Spain, Portugal, and the United Kingdom; the *CTX-M-2* group was mainly identified in South America and Japan, while in China the *CTX-M-9* group enzymes, in the United States or North Africa *CTX-M* group enzymes were common. *CTX-M-15* and *CTX-M-14* are the most detected ones as they are commonly detected in animal-based foods, humans, and clinically important food pathogens [13]. In Turkey, as in the rest of the world, antibiotic restrictions are emphasized and research continues. However, research on animal based products is limited.

In this study; phenotypic antibiotic resistance properties of ESβL, Amp-C, and carbapenemase-producing *E. coli* strains isolated and identified from red meats offered for retail sale were analyzed by E-Test and disc diffusion test. Antibiotic resistance genes were determined by PCR and LAMP PCR. In addition, the samples were pre-enriched in buffered peptone water (BPW) for 24 hours. The study, it is aimed to research the resistance genes by performing direct PCR and LAMP PCR without the isolation of the agent.

MATERIALS AND METHODS

Collection of red meat samples

One hundred and twenty samples (n:120) were taken from veal (n:62) and lamb (n:58) meats in one-week periods from retail butchers in Çorum and placed in sterile sample bags at 4°C. It was delivered to the laboratory in the cold chain as soon as possible. 100 samples were taken from the same sites for PCR and LAMP PCR analysis from BPW.

Isolation and identification

By the protocol of the European Union Reference Laboratory-Antimicrobial Resistance (EURL-AR), a 25 g sample was homogenized in 225 mL BPW, and then incubated for 18-22 hours at 37°C for pre-enrichment. To isolate ESβL producing strains, it was inoculated on Mac Conkey Agar containing cefotaxime (1mg/L) and incubated for 18-22 hours at 44°C. A loopful of samples was taken from the pre-enriched samples and inoculated on Mac Conkey Agar containing meropenem (0.5 µg/ml) for determination of carbapenemase activity and incubated for 18-22 hours at 44°C [14].

Antimicrobial susceptibility testing

To investigate the phenotypic resistance of the isolates with the Kirby-Bauer disc diffusion test; ampicillin (AM, 10 µg), azithromycin (AZM, 15 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (CN, 10 µg), meropenem (MEM, 10 µg), nalidixic Acid (NA 30 µg), tetracycline (TE 30 µg), tigecycline (TGC, 15 µg), trimethoprim/sulfamethaxol (SXT, 25 µg), cefoxitin (FOX, 30 µg), cefotaxime (CTX, 30 µg), cefotaxime/clavulanic 15 antibiotic discs including acid (CTC, 40 µg), ceftazidime (CAZ, 30 µg), ceftazidime/clavulanic acid (CZC, 40 µg) were used. In the E-Test used to determine the minimum inhibition value (MIC) of the isolates, meropenem (MP, 0.02-32 µg), imipenem (IMP, 0.02-32 µg), colistin (CO, 0.016-256 µg), cefoxitin (FX, 0.016-256 µg), cefotaxime (CT, 0.016-256 µg), cefotaxime/clavulanic acid (CT+CLA, 0.016-1 µg), ceftazidime (TZ, 0.016-256 µg), ceftazidime/clavulanic acid A total of 8 E-test strips (TZ+CLA, 0.064-4 µg) were used.

PCR primers and LAMP PCR primers

PCR primers [15] are given in Table 1 and LAMP PCR primers [16] are given in Table 2.

Table 1. *ESβL, Amp-C and carbapenemase genes primers for PCR [15].*

ESβL genes	**5`- 3` primer**
CTXM-9	(F) TCAAGCCTGCCGATCTGGT
	(R) TGATTCTCGCCGCTGAAG
CTXM-1	(F)TTAGGAARTGTGCCGCTGYA
	(R) CGATATCGTTGGTGGTRCCAT
SHV	(F) AGCCGCTTGAGCAAATTAAAC
	(R) ATCCCGCAGATAAATCACCAC
TEM	(F) CATTTCCGTGTCGCCCTTATTC
	(R) CGTTCATCCATAGTTGCCTGAC
OXA-1	(F) GGCACCAGATTCAACTTTCAAG
	(R) GACCCCAAGTTTCCTGTAAGTG
Amp-C gene	**5`- 3` primer**
FOX	(F) CTACAGTGCGGGTGGTTT
	(R) CTATTTGCGGCCAGGTGA
Carbapenemase genes	**5`- 3` primer**
KPC	(F) CATTCAAGGCTTTCTTGCTGC
	(R) ACGACGGCATAGTCATTTGC
NDM1	(F)GATGCCGACACTGAGCACTA
	(R)GGGCCGTATGAGTGATTGC
OXA-48	(F)GCTTGATCGCCCTCGATT
	(R) GATTTGCTCCGTGGCCGAAA
VIM	(F) GATGGTGTTTGGTCGCATA
	(R) CGAATGCGCAGCACCAG
IMP	(F) TTGACACTCCATTTACDG
	(R) GATYGAGAATTAAGCCACYCT

Table 2. *Carbapenemase genes primers for LAMP PCR [16].*

Genes names	Gene Bases
NDM1 F3	GCTTGCCCCGCAAGAG
NDM1 B3	AGCCACCAAAAGCGATGTC
NDM1 FIP	CGGTTGCTGGTTCGACCCATTTTGGTTGCGGCGCAACAC
NDM1 BIP	GCCCAACTTTGGCCCGCTCATTTTTGCCGTCGATCCCAACG
NDM1 FLP	GCGGCGAAAGTCAGGCT
NDM1 BLP	GGTATTTTACCCCGGCCCCG
KPC F3	TGGGCAGTCGGAGACAA
KPC B3	GTTGACGCCCAATCCCTC
KPC FIP	ATAGGTGCGCGCCCAGTGGTTTTCGGAACCTGCGGAGTGT
KPC BIP	CGTCTACACCCGGGCGCCTATTTTAGCGCGAGTCTAGCCG
KPC FLP	GGCATAGTCATTTGCCGTGC
KPC BLP	ACAAGGATGACAAGCACAGCG
OXA-48 like F3	AATAGCTTGATCGCCCTC
OXA-48 like	CCATAATCGAARGCRTGYAGC
OXA-48 like	GATTCCAAGYGGCGATATCRCTTTTGGCGTGGTTAAGGATGA
OXA-48 like	TAATYACCGCGATGAARTAYTCAGTTTTYTTRCTCATACGTG
OXA-48 like	TGTCCATCCCACTTAAAGACTTG
OXA-48 like	TGCCTGTTTATCAAGAATTTGCCCG
VIM F3	CCTGTAACGCGTGCAGTC
VIM B3	GCAGCACCAGGATAGAAGAG
VIM FIP	GATGCGTACGTTGCCACCCCTTTTTCCACGCACTTTCATGAC
VIM BIP	GGAACGAGATTCCCACGCACTTTTTTCTACTGGACCGAAGCG
VIM FLP	ACATCAACGCCGCCGAC
VIM BLP	CTCTAGAAGGACTCTCATCGAGCG
IMP F3	GCAGAGTCTTTGCCAGAT
IMP B3	GTCGCTATGAAAATGAGAGG
IMP FIP	GCCCCACCCGTTAACTTCTTTTTTGAAAAGCTTGATGAAGGC
IMP BIP	TGCTGAGGCTTACCTAATTGACACTTTTTATAGCCACGCTCC
IMP FLP	CAAACGAAGTATGAACATAA
IMP BLP	CGGCTAAAGATACTGAAAAGTTAG

E-test and Disk diffusion test

The isolate was adjusted in 5 ml physiological saline with a suspension density of 0.5 Mc Farland (BBL Mc Farland Turbidity Standard) (10^8 cfu/ml). Planted on the surface of Mueller Hinton Agar. Discs and E-Test strips were inserted with sterile forceps. Petri dishes were incubated at 37°C for 18-24 hours and the zones formed after incubation were measured with a ruler and recorded. Disk diffusion measurement results were investigated according to EUCAST (2021) [17] and CLSI [18] criteria. At the end of the incubation, the criterion by which the elliptical zone of inhibition cuts the strip in the E-Test reports the MIC result. E-Test zone diameters were evaluated according to EUCAST and CLSI criteria. It was evaluated according to EUCAST criteria specified in [17]. Those who could not meet the criteria in EUCAST were evaluated according to the CLSI (2014) criteria [18]. In addition, the multi-antibiotic resistance (Multy-Drug Resistance-MRD)

index was also calculated in the study; strains with an index greater than 0.2 were considered MDR positive if they showed resistance to more than 3 antibiotics [19].

DNA extraction

In 100 µl sterile distilled water, one colony of bacteria was taken and homogenized; It was heated at 95 °C for 10 minutes and at 10.000 rpm and 2 minutes. centrifuged. By taking the supernatant, the DNA measured by nano-drop spectrophotometer was measured as ng/mL [14]. In the study, from the samples in which *E. coli* was also detected, 1 g sample was inoculated into 9 ml of BPW and incubated for 18-22 hours at 37°C, and DNA was isolated directly from BPW. For enzyme-mediated isolation, 1 µl of the enzyme, 10 µl of buffer, and 20 µl of BPW of the relevant sample were taken into eppendorf tubes and 69 µl of sterile distilled water was placed on it, after completing the composition to 100 µl, at 75°C for 15 minutes and 95 DNAs were isolated by boiling for 5 minutes at 95°C. The isolated DNAs were measured by nano-drop spectrophotometer and subjected to PCR and LAMP PCR tests.

Determination of ESβL, Amp-C, and Carbapenemase genes by PCR

To determine bla$_{CTXM-9}$, bla$_{CTXM-1}$, bla$_{SHV}$, bla$_{TEM}$, bla$_{OXA-1}$ for ESβL activity in PCR test; Primers bla$_{FOX}$ were used for amp-C activity, bla$_{OXA-48}$, bla$_{IMP}$, bla$_{VIM}$, bla$_{KPC}$, bla$_{NDM-1}$ primers were used for carbapenemase activity. 25 µl PCR reaction volume in the PCR method; It was obtained from 12.5 µl master mix, 1 µl DNA, 5 µl primer, and 6.5 µl sterile distilled water. PCR protocol; The first denaturation was performed at 94°C for 10 minutes, denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and elongation at 72°C for 1 minute for 30 cycles. In the following; 7 min at 72°C. As a result, the protocol was completed [14]. 2% agarose gel was prepared for electrophoresis. The electrophoresis tank was filled with a sufficient amount of 1XTAE. After the loading was completed, it was subjected to electrophoresis at 100 Volts for 1 hour. The gel was placed in the gel imager device under UV light and the images of the gel were recorded.

Determination of carbapenemases by LAMP PCR

Only the presence of carbapenemase genes was analyzed in LAMP PCR. In the LAMP PCR method, the total reaction volume will be 25 µl; Prepared with 15 µl master mix, 5 µl DNA, and 5 µl primer. LAMP PCR steps; It was kept in the heated block at 65°C for 30 minutes and subjected to electrophoresis.

Statistical analysis

The KAPPA test evaluated the phenotypic and genotypic analysis results of the methods used in the study of ESβL, Amp-C, and carbapenemase genes. The results were evaluated according to Mc Hugh (2012) [20].

RESULTS AND DISCUSSION

Isolation and identification

E. coli isolates suspected of ESβL, Amp-C, and carbapenemase producing was isolated in 14 of 120 samples (62 calves and 58 lambs). As a result of identification with Gram stain, oxidase test, and BBL crystal rapid test system, 12 (5 calves and 7 lambs) were

found to be *E. coli*. While 11 isolates were obtained in Mac Conkey Agar containing cefotaxime, only 1 isolate was detected (OXA 18) in Mac Conkey Agar containing meropenem.

Disk diffusion test results

MDR was determined in 91.6% of the isolates phenotypically. Ampicillin and tetracycline 91.6%, cefotaxime 83.33%, chloramphenicol, nalidixic acid, and ciprofloxacin 75%, azithromycin and sulfamethoxole/trimethoprim 66%, gentamicin and ceftazidime 41.6%, and tigecycline 33.3% resistance was observed. The antibiotics used in disc diffusion and their results are shown in Table 7. ESβL resistance was detected for CTX-CA (cefotaxime/clavulanic acid) in 9 isolates (4 isolates from lamb, 5 isolates from veal), and for CAZ-CA (ceftazidime/ clavulanic acid) in 1 isolate (from lamb). All isolates (5 lambs and 7 veal) were found to be resistant to cefoxitin, which was used to determine Amp-C resistance. No isolates were resistant to meropenem used for carbapenemase resistance; however, 2 isolates were detected as intermediate (I) (Table 3).

Tablo 3. *Disc diffusion test results.*

	Meats	**Beef**	**Beef**	**Lamp**	**Beef**	**Lamp**	**Beef**	**Lamp**	**Beef**	**Lamp**	**Lamp**	**Lamp**	**Lamp**
	Isolates	**1D**	**7C**	**7D**	**9**	**12A**	**12B**	**13A**	**14D**	**18**	**OXA 18**	**22B**	**24D**
Antibiotics	**AM**	0/R	0/R	0/R	0/R	0/R	0/R	0/R	0/R	0/R	14/S	0/R	0/R
	AZM*	0/R	25/S	27/S	24/S	20/S	0/R	0/R	0/R	0/R	0/R	8/R	0/R
	C*	27/S	0/R	30/S	0/R	0/R	0/R	0/R	0/R	0/R	30/S	0/R	0/R
	CIP	22/R	27/S	25/S	12/R	9/R	0/R	12/R	10/R	9/R	35/S	9/R	17/R
	GM	24/S	13/R	24/S	20/S	23/S	0/R	20/S	10/R	0/R	21/S	0/R	21/S
	MEM	25/S	22/S	23/S	22/S	24/S	30/S	22/S	23/S	23/S	25/S	21/-	20/-
	NA*	18/I	20/S	0/R	0/R	0/R	0/R	0/R	0/R	0/R	21/S	0/R	0/R
	TE*	0/R	0/R	0/R	0/R	0/R	0/R	0/R	0/R	0/R	13/I	0/R	0/R
	TGC	17/R	18/S	17/R	18/S	19/S	30/S	20/S	20/S	16/R	18/S	18/S	17/R
	SXT	0/R	23/S	0/R	0/R	0/R	0/R	0/R	30/S	0/R	30/S	0/R	19/S
	FOX	8/R	0/R	0/R	16/R	10/R	01/R	11/R	18/R	0/R	10/R	0/R	0/R
	CTX*	8/R	22/R	13/R	9/R	0/R	0/R	8/R	11/R	25/S	35/S	0/R	20/R
	CTX/ CA	30 ESβL	30 ESβL	30 ESβL	30 ESβL	30 ESβL	55 ESβL	30 ESβL	31 ESβL	27	35	26 ESβL	23
	CAZ*	19/I	16/R	25/S	22/S	16/R	43/S	17 R	21/S	21/S	30/S	16R	13/R
	CAZ/ CA	18	18	21	17	15	0	16	20	11	27	12	0 ESβL

*******: CLSI criteria, -: Not detected, **R**: Resistant, **S**: Susceptible, **I**: Intermediate, **ESBL**: ESBL positive isolates*

E-test results

While no resistance was found against CTX-CA (cefotaxime/ clavulanic acid), which is used to determine ESβL activity, resistance was found in 1 isolate to CAZ-CA (ceftazidime/clavulanic acid). Amp-C and Carbapenemesis activity could not be detected in any isolate (Table 4).

Table 4. *E-Test results.*

	Meats	Beef	Beef	Lamp	Beef	Lamp	Beef	Lamp	Beef	Lamp	Lamp	Lamp	Lamp
	Isolates	**1D**	**7C**	**7D**	**9**	**12A**	**12B**	**13A**	**14D**	**18**	**XA18**	**22B**	**24D**
Antibiotics	**CO**	0,75/S	0,75/S	0,75/S	0,75/S	24/R	0,75/S	0,75/S	0,75/S	-	0,75/S	0,75/S	0,75/S
	MP	0,023/S	0,032/S	0,032/S	0,032/S	0,032/S	0,002/S	0,047/S	0,032/S	0,023/S	0,016/S	0,032/S	0,047/S
	IMP	0,125/S	0,094/S	0,094/S	0,094/S	0,125/S	-	0,094/S	0,094/S	0,064/S	0,032/S	0,094/S	0,094/S
	FX*	3/S	24/S	2/S	1/S	16/I	12/S	1,5/S	1/S	-	2/S	3/S	-
	CT	-	1,25/S	-	-	-	-	-	-	1/S	0,125/S	-	6/R
	CT/CA	1,19	1,032	1,064	1,01	1,125	1,032	1,0123	1,094	-	1,042	1,064	-
	TZ	3/S	3/S	1,75/S	3/S	5/R	4/S	8/R	2/S	4/S	1,50/S	0/S	12/R
	TZ/ CA	1,19	1,064	1,125	1,06	1,19	1,094	1,064	1,19	1	1,125	1,094	1,5 ESβL

**:CLSI criteria, -: Not detected, R: Resistant, S: Susceptible and I: Intermediate, ESBL: ESBL positive isolates*

PCR and LAMP PCR results

When resistance genes were investigated by PCR and LAMP PCR, the *CTX-M1* gene was detected in 4 isolates (12B, 1D, 12A, 9D,) and the *SHV* gene was detected in all isolates; the carbapenemase gene and Amp-C gene were not detected (Table 5). In PCR and LAMP PCR results from samples taken directly from BPW (n=10), *CTX-M1* gene was detected in 4 samples; It was observed that the *SHV* gene was detected in 10 samples (Table 6) from BPW. All carbapenemase genes were detected in 2 samples by LAMP PCR from BPW. The comparison of the tests applied in this study is shown in Table 7.

Table 5. *PCR and LAMP PCR ESβL, Amp-C, and carbapenemase genes results*

Isolates	ESβL Genes (PCR)					Amp-C Gene (PCR)	Carbapenemase Genes (PCR/LAMP PCR)				
	CTX-M1	CTX-M9	SHV	TEM	OXA-1	FOX	NDM	OXA-48	IMP	VIM	KPC
1D	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
7C	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
7D	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
9D	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
12A	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
12B	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
13A	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
14D	-	-	+	-	-	-	-/+	-/+	-/+	-/+	-/+
18	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
OXA18	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
22B	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
24D	-	-	+	-	-	-	-/+	-/+	-/+	-/+	-/+

Table 6. *PCR and LAMP PCR test results from BPW*

	ESβL genes (PCR)					Amp-C Gene (PCR)	Carbapenemase genes (PCR/LAMP PCR)				
Isolates	**CTX-M1**	**TX-M9**	**SHV**	**TEM**	**OXA-1**	**FOX**	**NDM**	**OXA-48**	**IMP**	**VIM**	**KPC**
1D	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
7C	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
7D	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
9D	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
12A	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
12B	+	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
13A	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
14D	-	-	+	-	-	-	-/+	-/+	-/+	-/+	-/+
22B	-	-	+	-	-	-	-/-	-/-	-/-	-/-	-/-
24D	-	-	+	-	-	-	-/+	-/+	-/+	-/+	-/+

Tablo 7. *The comparison of the tests applied in the study*

Tests	**DISC DIFFUSION**	**E-TEST**	**PCR (ESβL)**	**PCR (CARBA)**	**LAMP PCR**	**BPW PCR**	**BPW LAMP PCR**
12B	**ESβL+Amp-C**		**ESβL**			**ESβL**	
1D	**ESβL +Amp-C**		**ESβL**			**ESβL**	
12A	**ESβL +Amp-C**		**ESβL**			**ESβL**	
9D	**ESβL +Amp-C**		**ESβL**			**ESβL**	
7D	**ESβL +Amp-C**		**ESβL**			**ESβL**	
7C	**ESβL +Amp-C**		**ESβL**			**ESβL**	
24D	**ESβL +Amp-C**	**ESβL**	**ESβL**		**Carba**	**ESβL**	**Carba**
14D	**ESβL +Amp-C**		**ESβL**		**Carba**	**ESβL**	**Carba**
13A	**ESβL +Amp-C**		**ESβL**			**ESβL**	
22B	**ESβL +Amp-C**		**ESβL**			**ESβL**	
OXA18	**Amp-C**		**ESβL**				
18	**Amp-C**		**ESβL**				

ESβL*: ESβL activities positive,* ***ESβL*** *+Amp-C: ESβL +Amp-C activities positive, Amp-C: Amp-C activities positive,* ***Carba****: Carbapenemase activities positive*

Statistical results

The results of the Kappa test performed in the SPSS program were evaluated (Table 8). Minimal agreement between disc diffusion and PCR (ESβL), almost perfect agreement between disc diffusion and PCR (*CTXM1*-ESβL), the almost perfect agreement between disc diffusion and PCR (*SHV*-ESβL) was found, while other comparisons found no agreement. In terms of genes, it is remarkable that there is an

almost perfect agreement between the results of the disk diffusion test and the PCR results.

Tablo 8. *Kappa test results*

Test	K	Evaluation
DD and E-Test (ESβL)	0,05	No agreement
DD and PCR (ESβL)	0,32	Minimal agreement
DD and PCR (*CTXM*1-ESβL)	**1,52**	**The almost perfect agreement**
DD and PCR (*SHV*-ESβL)	**1**	**The almost perfect agreement**
DD and E-Test (Amp-C)	0	No agreement
DD and LAMP PCR (Carba)	0	No agreement
E-Test and PCR (ESβL)	0	No agreement
E-Test and PCR (*CTXM-1*-ESβL)	0,05	No agreement
E-Test and PCR (*SHV*-ESβL)	0	No agreement
E-Test and PCR (Amp-C)	0	No agreement
E-Test and LAMP PCR (Carba)	0	No agreement

In the world, 63,000 tons of antibiotics are used in the livestock sector; to use of antibiotics is expected to increase to 105,000 tons in 2030 [21]. Diseases caused by drug-resistant *Enterobacteriaceae* are increasing. In particular, resistance to β-lactam antibiotics poses a significant risk in human and veterinary medicine.

Amp-C positivity was found in all 12 *E. coli* isolates by disk diffusion method; however, Amp-C positivity was not found with the E-Test method. Genotypically, Amp-C (bla*FOX*) gene was not found in PCR. In our study, Amp-C activity may have been expressed by a different gene than the investigated bla$_{FOX}$ gene. Carbapenemase activity; in disk diffusion, E-Test, and PCR; was not detected in any sample; On the other hand, in LAMP PCR, isolates 14D and 24D were positive. All bla*NDM*, bla*OXA-48*, bla*IMP*, bla*VIM*, and bla*KPC* genes were found in both isolates. The presence of carbapenemase genes in LAMP PCR can be explained by the fact that this method is more specific than PCR [22].

In a study conducted in Amasya [23], by our study, the ESβL ratio of *Enterobacteriaceae* was determined as 68%, *CTX-M* type at 35.29%, and *SHV* type β-lactamase at 23.52%. The *TEM* type β-lactamase was not determined in any of the isolates. In another study, ESβL was detected phenotypically in 23 (20.9%) in 110 raw red meat samples (65 calves and 45 sheep) collected from Bursa, Tekirdağ and Yalova [24]. The rate of ESβL producing *E.coli* isolation was 10%, in our study. In a study investigating the presence of ESβL and/or Amp-C producing *E. coli* in chicken and beef meats offered for retail sale; 7 cefotaxime (2 µg/mL) resistant *E. coli* isolates (n = 100) from red meat samples were found; It was stated that all of them showed ESβL feature by the disk combination method. While bla*CTX-M-3*, bla*CTX-M-15*/bla*TEM-1b*, and bla*TEM-1b*/ bla*CMY-2* genes were found in 3 isolates; In the 4 phenotypic ESβL-positive strains, however, they could not find the genes tested. Cefuroxime (85.7%), nalidixic acid (85.7%), tetracycline (100%), streptomycin (100%), and trimethoprim-sulfamethoxazole resistance was relevealed in ESβL-producing *E. coli* isolated from beef (n: 7/100) (85.7%). All isolates were detected to be susceptible to amikacin, imipenem, and

cefepime. Likewise, no resistance to imipenem was found in our study either; Tetracycline, nalidixic acid, and trimethoprim-sulfamethoxazole resistance were highly prevalant among isolates [25].

In a study conducted in Egypt, it was determined that 17% of 100 samples obtained from retail mutton were ESβL producing *E. coli*. When examined genotypically, *bla*$_{CTX-M}$, bla$_{CTX-M-15}$, bla$_{CTX-M-14}$, bla$_{TEM}$, and bla$_{SHV}$ were found [26]. In a study conducted in Iran [27], the most common resistance gene in *E. coli* and *Salmonella* spp. isolates were bla$_{TEM}$ (44.11%) in samples from raw kebab and hamburger. In addition, *E. coli* in 14 isolates (23.72%) and *Salmonella* spp. in 10 isolates (29.41%) were found to have the bla$_{SHV}$ gene. In a study conducted in Ghana [28], 4 *E. coli* isolates of 98 *E. coli* isolates obtained from raw meat were determined to contain the bla$_{TEM}$ gene, and *E. coli* isolates were found to have the bla$_{TEM}$ gene. Most of them revealed phenotypic resistance against ampicillin, amikacin, tetracycline, sulfometaxole/trimethoprim, and cefuroxime. The bla$_{SHV}$ and bla$_{CTX-M}$ genes were not detected in the isolates. Similarly, in our study, the antibiotics that showed the highest resistance were ampicillin and tetracycline (91.6%) [29].

In the USA, 7 *CTX-M*-type ESβL genes have been reported from 113 ESβL *E. coli* obtained from sheep and abattoir environments in North Carolina [30]. It was emphasized that the dominant gene was the *CTX-M1* gene. Among the other β-lactamase genes detected, *TEM-1* (46.9%), *CARB-2* (14.2%) and Amp-C β-lactamase gene, were *CMY-2* (% 9.7). Almost all isolates (98.2%) were identified as multidrug resistant. Compared to our study, the *CTX-M1* gene is the second dominant gene in our study. Multiple antibiotic resistance is present in almost all of our isolates.

Solanki et al. (2013)[31] used LAMP test to detect bla$_{NDM-1}$ and bla$_{KPC}$ genes. LAMP assay has been reported to detect one or both of the bla$_{NDM-1}$ and bla$_{KPC}$ genes in four isolates missed by PCR. Compared to our study, the carbapenem resistance that we could not detect phenotypically and in PCR was determined by LAMP PCR.

CONCLUSION

As a result, a high rate of multi-drug resistance (91.6%) against *E. coli* was detected in our study of red meats. According to the protocol of EURL-AR, ESβL, Amp-C, and carbapenemase activities of *E. coli* isolated from red meat for the first time were examined and a high rate of ESβL activity was found. According to the statistical results of our study; The relationship between the results of the disc diffusion test and PCR tests was found to be significant. The LAMP PCR test was found to be more sensitive in tests performed for carbapenemase activity. The fact that there is a positive sample for all genes in terms of carbapenemase activity indicates that carbapenemase activity may pose a risk in the future. When examined based on genes, it was found that *SHV* and *CTX-M1* genes are common in our country. In addition, it was concluded that resistance genes could be detected after a 1-day pre-enrichment with BPW, and therefore, resistance genes could be tested in a shorter time.

Antibiotic resistance genes show an increasing trend in Turkey, although at a lower rate than in other countries. Taking the necessary measures to prevent antibiotic resistance is very important within the scope of one health concept.

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