Investigation of Broad-Spectrum Beta-Lactamase Production, Antibiotic Resistance and *mcr*-1 Gene in *Escherichia coli* Isolates from Broilers

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ABSTRACT

The detection of extended-spectrum β-lactamase (ESBL) production and mobilized colistin resistance gene (*mcr*) in *Escherichia coli* isolates reveals the potential for developing bidirectional resistance to both extended-spectrum β-lactamases and critically important colistin. The risk of transmission of these resistant bacteria from animals to humans can cause serious difficulties in terms of public health and veterinary medicine. This study was aimed to investigate the antibiotic resistance profiles of ESBL-producing *E. coli* isolates obtained from broilers with colibacillosis in the western part of Türkiye, the main ESBL genotypes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$) and the *mcr*-1 gene, one of the important genetic determinants of colistin resistance. In the study, 247 (75%) *E. coli* isolates obtained by classical conventional methods from 327 colibacillosis suspected broiler livers brought to the laboratory for routine diagnosis within the scope of various studies during the last year were used. Bacterial identification was done by classical conventional methods. ESBL-producing isolates were phenotypically confirmed with CHROMagar™ ESBL. Genotypes and *mcr*-1 genes of ESBL-producing isolates were examined by polymerase chain reaction. Antibiotic resistance profiles of the isolates against 20 antibiotics belonging to nine antimicrobial families were evaluated by automated microbiology system (BD Phoenix, Becton-Dickinson, USA). Isolates resistant to at least one antibiotic from three or more antibiotic classes were considered as multidrug resistant (MDR). ESBL prevalence in isolates was determined as 27% (66 isolates). The most common ESBL gene was *bla*$_{TEM}$ (53%), and *bla*$_{CTX-M}$ (27%), *bla*$_{SHV}$ (8%) and *bla*$_{OXA}$ (5%) genes were also detected. All ESBL-producing isolates were determined to be MDR. All of the isolates were resistant to tigecycline, 97% to ampicillin and 91% to ciprofloxacin. The highest susceptibility was observed against amikacin, ertapenem, imipenem and meropenem (100%). In addition, the *mcr*-1 gene was detected in 12% of the ESBL-producing isolates. These results showed that ESBL production was high in *E. coli* isolates obtained from broilers, the *bla*$_{TEM}$ genotype was more dominant than other genotypes and the *bla*$_{TEM}$ genotype showed an increasing prevalence. The fact that all ESBL-producing isolates were MDR displayed the difficulty of treating these bacteria. The coexistence of ESBL and plasmid-mediated colistin resistance genes revealed that these bacteria pose a serious risk to public health.

Keywords: ESBL; *Escherichia coli*; broiler; antimicrobial resistance ESBL; *mcr*-1.

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to beta-lactam antibiotics, including third-generation cephalosporins and monobactams. These enzymes inactivate antibiotics by hydrolyzing the beta-lactam ring. ESBLs are found primarily in Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*, making infections caused by these bacteria difficult to treat (1). This resistance mechanism has become more widespread with increased contact between human and animal populations (2). This increases the risk of transmission of resistant bacteria to humans between humans and animals sharing the same environment (3).

To date, more than 350 ESBL variants are known, which have been classified into nine distinct structural and evolutionary families (TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA) based on amino acid sequence comparisons. The main types of ESBL variants include TEM, SHV, CTX-M, and OXA (4). The *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genotypes have traditionally been the most common, but the *bla*$_{\text{CTX-M}}$ genotype has been observed to be more widespread in recent years (5). The *bla*$_{\text{TEM}}$ gene was first isolated from a patient named Temoneira and generally confers resistance to broad-spectrum beta-lactam antibiotics such as ampicillin and piperacillin (6). The *bla*$_{\text{CTX-M}}$ gene, called cefotaximase-Munich, confers high-level resistance to third-generation cephalosporins (e.g., cefotaxime and ceftazidime) (7). *bla*$_{\text{SHV}}$ genes often produce enzymes called sulfhydryl variants. This gene confers resistance to beta-lactam antibiotics such as cephalosporins and penicillins (8). Oxacillinase genes (*bla*$_{\text{OXA}}$) often produce enzymes that confer resistance to carbapenems and broad-spectrum cephalosporins (9). Most genes encoding ESBLs are plasmid-borne and are often found in transposons and integrons, facilitating their mobilization by other resistance determinants. Therefore, genes encoding ESBLs can be easily transferred between bacteria (1).

It is known that poultry and poultry products are a potential source of antibiotic-resistant bacteria, including ESBL-producing *E. coli*, to humans (10). Therefore, the presence of ESBL-producing bacteria in poultry may pose a serious threat to public health. These bacteria can be transmitted to humans through direct contact or through contaminated food products (11). In Africa, 20% of ESBL-producing *E. coli* with *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes have been detected in chicken (12). Comparative analysis in Central Europe has shown genetic similarity of ESBL-producing *E. coli* from Mongolian migratory birds and clinical isolates from European hospitals (13). In Egypt, at least one ESBL phenotype/gene was phenotypically and genotypically identified in 47% of 120 chicken farms and in all human samples, and a high incidence of *bla*$_{\text{CTX-M}}$ gene was detected in chicken isolates (14).

In Türkiye, a limited number of studies have been conducted on the presence of ESBL-producing *E. coli* isolates in broilers, and these studies were conducted with samples taken from healthy birds. First, in 2017, the prevalence of ESBL in *E. coli* isolates obtained from cloacal swabs of healthy broilers was determined as 8.3%, and the *bla*$_{\text{CTX-M}}$ gene was reported in 80% of the isolates (15). In recent studies, ESBL-producing *E. coli* was reported in *E. coli* isolates obtained from chicken meat (16) and healthy pigeon cloacal swabs (17).

This study aimed to investigate the antibiotic resistance profiles of ESBL-producing *E. coli* isolates obtained from broilers with colibacillosis in western Türkiye, the main ESBL genotypes (*bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{CTX}}$), and the *mcr*-1 gene, which is one of the most important genetic determinants of colistin resistance.

MATERIALS AND METHODS

Materials

The liver samples of 327 broilers belonging to the Ross 308 breed, aged between 1 and 40 days, brought to the laboratory with suspicion of colibacillosis and obtained from farms belonging to the same broiler integration company were used as material in this study. The main clinical symptoms observed in these broilers were recurrent cough, anorexia, dyspnea, diarrhea, weight loss and lameness. The minimum sample size used in the study was calculated as 203 with an estimated prevalence of 75% (based on the studies of Aberkane and Seferoğlu) (18, 19), a confidence level of 90% and a margin of error of 5% (20).

Bacterial isolation and identification

Isolation was performed using standard bacteriological methods. Samples were streaked onto MacConkey Agar (Merck 105465, Germany) and incubated aerobically at 37°C for 24 hours. The next day, a single lactose-positive colony on MacConkey agar was sub-cultured onto EMB agar (Merck

101347, Germany). After another 24 hours of incubation at 37°C, *E. coli* showing characteristic green metallic sheen colonies were collected. These colonies were subjected to biochemical tests (oxidase test, motility, citrate utilization, indole test, methyl red, etc.) (21). For the confirmation of bacterial identification, an automated system (BD Phoenix, Becton-Dickinson, USA) was used for evaluation according to the manufacturer's instructions. The isolates were stored in Brain Heart Infusion Broth (BHIB) containing 20% glycerol (Merck 110493, Germany) at -20°C.

ESBL-producing *E. coli* isolation

ESBL-producing *E. coli* isolates were identified using ChromagarTm ESBL agar (France, Chromagar, 201470) according to the manufacturer's instructions. Each *E. coli* isolate was incubated at 37°C for 18-24 hours under aerobic conditions. After incubation, bacteria forming pink (to burgundy) colonies on ChromagarTm ESBL agar were considered ESBL-producing *E. coli* strains. The colonies with pink colour were tentatively identified as ESBL producer. The ESBL producing isolates were further verified by Double-Disc Synergy Test (DDST) using ceftazidime (CAZ-30 µg) and ceftazidime with clavulanic acid (CAC-30/10 µg) as well as cefotaxime (CTX, 30 µg) and cefotaxime-clavulanate (CEC, 30/10 µg) discs. Briefly, *E. coli* isolates were inoculated into nutrient broth (Merck 105443, Germany) and incubated at 37°C. The bacterial culture with a turbidity equivalent to 0.5 Mac Farland standard unit was inoculated on to Mueller Hinton Agar (MHA) (Merck 103872, Germany) plates by spread plate method. The antibiotic discs were placed on the inoculated MHA plates at a distance of 20 mm apart and incubated overnight at 37°C. The inhibition zone diameter was measured for each antibiotic disc and its respective clavulanic acid containing discs. A difference of ≥5 mm in the presence of clavulanic acid when compared to its absence was considered as positive for the production of ESBL (22).

Antibiotic susceptibility test

Antibiotic susceptibility testing (AST) of the isolates identified as *E. coli* was performed using the BD Phoenix (Becton-Dickinson, USA) automated system with NMIC/ID 433 panels. The isolates were tested against 20 antibiotics belonging to nine different antimicrobial families (Lipopeptide: amikacin (AN), gentamicin (GM); Carbapenem: ertapenem (E), imipenem (IPM), meropenem (MEM); Cephem: cefazolin (CFZ), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP); Penicillin: ampicillin (AMP); Beta Lactam: ceftolozane-tazobactam (CT), amoxicillin clavulanate (AMC), ampicillin sulbactam (AS), piperacillin-tazobactam (PT); Lipopeptide: colistin (COL); Folate: trimethoprim-sulfamethoxazole (TS); Quinolone: ciprofloxacin (CIP), levofloxacin (LF), Tetracycline: tigecycline (TIG)). The resistance status of the isolates against these antibiotics was examined. The results were evaluated according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (23). *E. coli* ATCC 25922 strains were used as quality control organisms.

Phenotypic detection of colistin resistance

During the study, colistin resistance in all isolates was examined using an automated system. The interpretation of colistin minimum inhibitory concentration (MIC) results was carried out using the EUCAST clinical breakpoints (susceptible ≤ 2 mg/L; resistant > 2 mg/L) (23).

Multidrug resistance (MDR)

MDR was defined as resistance to three or more antimicrobial classes (24).

DNA extraction

In this study, DNA extraction was performed using a commercial genomic DNA extraction kit (InstaGene™ Matrix, Biorad, Dubai) following the manufacturer's instructions. DNA purity and quantity were assessed using a nanodrop spectrophotometer (Maestrogen, Taiwan). An OD260/OD280 ratio of 1.6-2.0 indicated sufficient DNA purity (25).

Polymerase chain reaction

PCRs were performed in a volume of 25 µl. The final concentrations were adjusted as follows: 1x of 10xTaq enzyme buffer solution, 2 mM of 25 mM $MgCl_2$, 0.2 mM of 10 mM dNTP, 0.4 ρmol of each primer, and 1.5 U of Taq DNA polymerase (Fermentas, Massachusetts, USA). After preparing the master mixes, PCR tubes were numbered according to the number of samples, and 22 µl of master mix with 3 µl of DNA was added for each sample. PCR pre-denaturation was performed at 95°C for 5 minutes, denaturation at 95°C

Table 1. Primers used in the study.

Primer	Target Gene	Sequence (5'-3')	Amplicon size (bp)	Annealing (°C)	Referans
usp-F	*usp*A	CCGATACGCTGCCAATCAG	884	59	26
usp-R		ACGCAGACCGTAGGCCAGAT			
SHV-F	*bla*SHV	CGCCTGTGTATTATCTCCCT	293	57	27
SHV-R		CGAGTAGTCCACCAGATCCT			
CTX-M-F	*bla*CTX	CGCTGTTGTTAGGAAGTGTG	569	57	27
CTX-M-R		GGCTGGGTGAAGTAAGTGAC			
TEM-F	*bla*TEM	ATAAAATTCTTGAAGACGAAA	1080	49	28
TEM-R		GACAGTTACCAATGCTTAATCA			
OXA-F	*bla*OXA	ACCAGATTCAACTTTCAA	598	47	29
OXA-R		TCTTGGCTTTTATGCTTG			
*mcr*1-F	*mcr*-1	CGGTCAGTCCGTTTGTTC	309	55	30
*mcr*1-R		CTTGGTCGGTCTGTAGGG			

for 30 seconds, then annealing at temperatures dependent on the specific primers (Table 1) for 30 seconds and an extension step at 72°C for 30 seconds, all steps for 30 cycles and a final extension at 72°C for 10 minutes.

On electrophoresis, a 2% agarose gel stained with Safe View (100 ml/6 µl) (ABM, Richmond, Canada) was used and the gel was exposed to 100 volts for 45 minutes. After electrophoresis, the gel was placed in the chamber of the transilluminator device which was connected to the computer and photographed under UV light (Vilbert Lourmat, Collegien, France). When the amplified product formed a band of the expected size (Table 2.), it was assumed to carry the gene examined.

Molecular Identification of *E. coli* Isolates

In order to perform molecular verification of *E. coli* isolates at species level, the presence of the universal stress protein gene 'uspA' in the isolates was examined by PCR (26). In molecular studies, *E. coli* ATCC 35150 was used as positive control and *Salmonella* Typhimurium ATCC 14028 as negative control.

Sequencing of PCR Products

In phenotypically ESBL producing isolates, the presence of *bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX genes was examined by PCR. In order to confirm the accuracy of the subtypes of the detected bla genes, the PCR product of a sample that gave a positive result from each gene subtype was purified and sent to Macrogen Company (Netherlands) for sequencing analysis. Sequencing results were analyzed using the BLAST program (www.ncbi.nlm.-nih.gov/BLAST). Sequences showing more than 97% homology were accepted as the detected gene types and used as positive control in PCR.

Genotypic Detection of Colistin Resistance

The presence of the *mcr*-1 gene, which is a plasmid colistin resistance gene, was examined in phenotypically colistin resistant isolates. In PCR procedures, *E. coli* NCTC 13846 strain was used as a positive control and *E. coli* ATCC 25922 strain was used as a negative control.

RESULTS

Bacterial isolates

In this study, 327 broiler samples suspected of colibacillosis were analyzed using an automated microbiology system (BD Phoenix, Becton-Dickinson, USA) and *E. coli* was identified in 247 (75%) of them. 27% of these isolates (66 isolates) were detected as ESBL positive with ChromagarTm ESBL agar. In all isolates, an amplification product of 884 bp was obtained in PCR analysis performed with uspA specific primers and the isolates were confirmed as *E. coli* (Figure 1). Then, ESBL resistance genes and antibiotic resistance profiles of 66 ESBL positive isolates were examined.

Characterization of ESBL-producing *E. coli* isolates

ESBL prevalence in isolates was detected as 27% (66 isolates). The most common ESBL gene was *bla*TEM (53%),

Table 2. Antibiotic resistance status of ESBL-producing *E. coli* isolates.

Antimicrobial Family-Antibiotic Name	ESBL positive isolates (n=66)			
	R	(%)	S	(%)
Aminoglycoside				
Amikacin	0	(0)	66	(100)
Gentamicin	20	(30)	44	(67)
Carbapenem				
Ertapenem	0	(0.0)	66	(100)
Imipenem	0	(0.0)	66	(100)
Meropenem	0	(0.0)	66	(100)
Cephem				
Cefazolin	48	(73)	16	(24)
Cefuroxime	53	(80)	11	(17)
Ceftazidime	34	(51)	29	(44)
Ceftriaxone	32	(48)	33	(50)
Cefepime	30	(45)	34	(52)
Penicillin				
Ampicillin	64	(97)	2	(3)
Beta Lactam				
CeftolozaneTazobactam	8	(12)	58	(86)
Amoxicillin Clavulanate	59	(89)	7	(11)
Ampicillin Sulbactam	52	(79)	14	(18)
Piperacillin Tazobactam	7	(11)	59	(84)
Lipopeptid				
Colistin	8	(12)	58	(88)
Folate				
Trimethoprim Sulfamethoxazole	54	(82)	12	(15)
Quinolone				
Ciprofloxacin	60	(91)	6	(9)
Levofloxacin	57	(86)	9	(11)
Tetracycline				
Tigecycline	66	(100.0)	0	(0.0)

while *bla*$_{\text{CTX-M}}$ (27%), *bla*$_{\text{SHV}}$ (8%) and *bla*$_{\text{OXA}}$ (5%) genes were also detected (Figure 2).

Antimicrobial resistance and multiple antibiotic resistance

In this study, antibiotic resistance profiles of 66 ESBL-producing *E. coli* isolates were evaluated using an automated microbiology system. All isolates were found to be susceptible to amikacin, ertapenem, imipenem, and meropenem, while they were found to be resistant to tigecycline (Table 2, Figure 3). It was determined that the isolates showed low-level resistance (11%-30%) to some antibiotics (gentamicin, ceftolozane-tazobactam, piperacillin-tazobactam, colistin), moderate resistance (31%-75%) to some antibiotics (cefazolin, ceftazidime, ceftriaxone, cefepime) and high-level resistance (76%-99%) to several antibiotics (cefuroxime, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin).

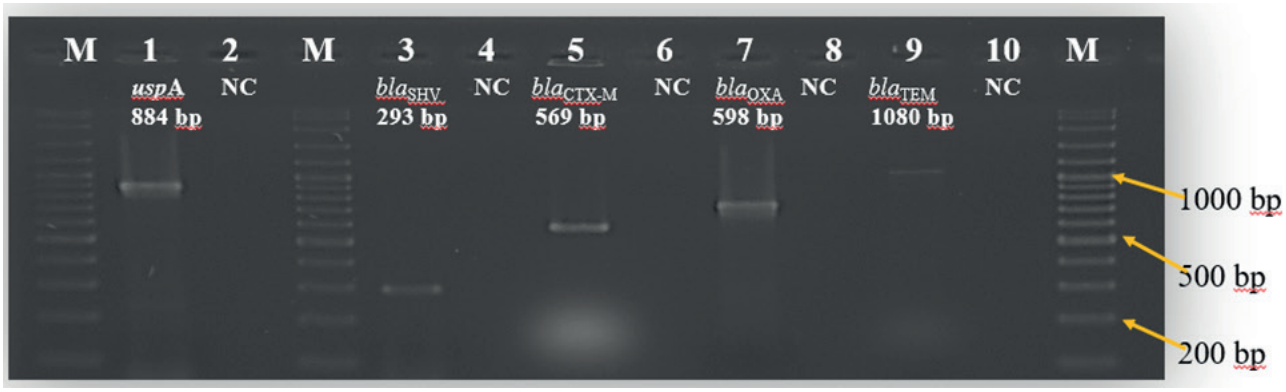

Figure 1. Agarose gel electrophoresis images of PCR products belonging to specific genes **1.** *usp*A gene (884 bp, *E. coli* ATCC 35150) **2.** NC (*S.* Typhimurium ATCC 14028) **3.** *bla*SHV gene (293 bp) **5.** *bla*CTX gene (569 bp) **7.** *bla*OXA (598 bp) **9.** *bla*TEM gene (1080 bp) **4, 6, 8, 10:** NC (DNA-free master mix). **M:** 100bp DNA Ladder (100 bp, Vivantis, Malaysia).

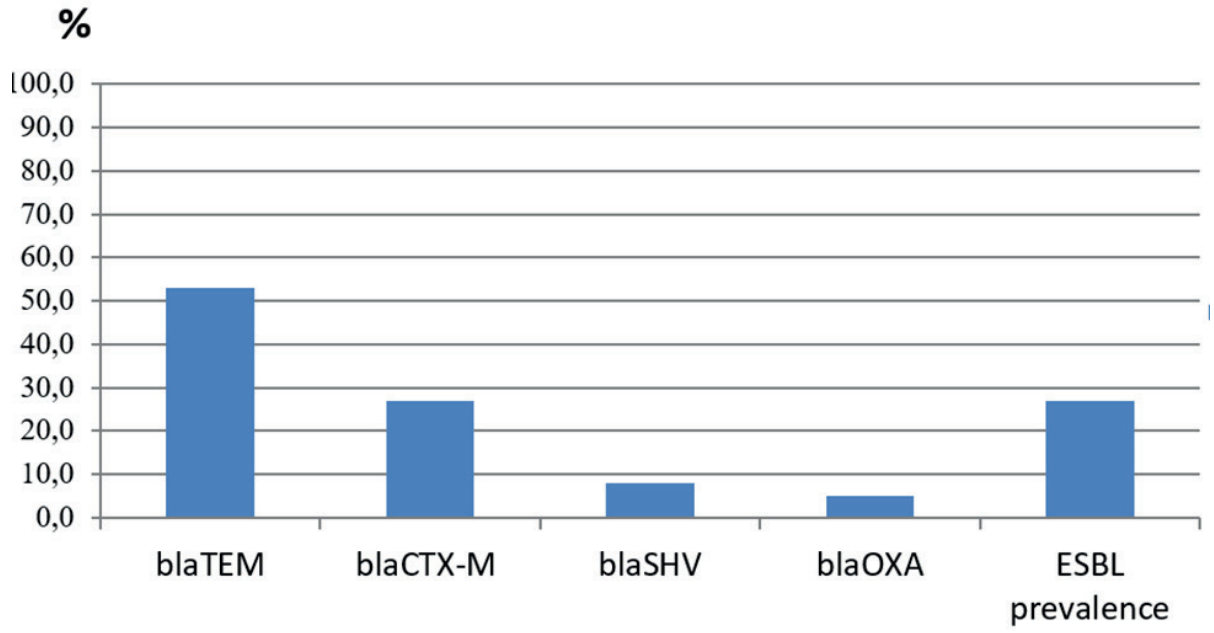

Figure 2. ESBL genotypes and prevalence.

Colistin resistance

In this study, the *mcr*-1 gene was detected in 12% of ESBL-producing isolates. It was determined that all isolates determined to be phenotypically resistant to colistin also carried the *mcr*-1 gene genotypically (Figure 4). These findings indicated that all phenotypically colistin-resistant isolates had the plasmid-mediated *mcr*-1 gene.

These results indicated that ESBL-producing *E. coli* strains developed resistance to a wide range of antibiotics and therefore treatment options should be carefully evaluated.

DISCUSSION

Detection of ESBL production is of great importance because ESBL-positive strains have been associated with higher mortality rates compared to ESBL-negative strains (1). ESBL-producing bacteria are often multidrug resistant, which significantly limits treatment options (31). The spread of these strains can occur both through direct animal contact and through consumption of contaminated animal products, which increases the risk of zoonotic transmission (32). In our study, the prevalence, antibiotic resistance profiles, and significant ESBL and colistin resistance genotypes of ESBL-

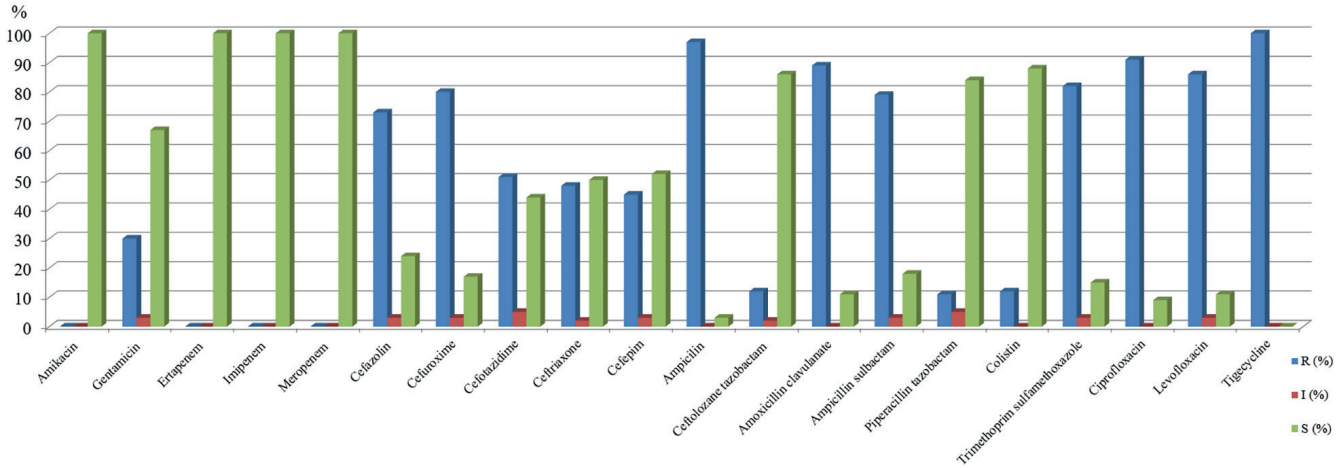

Figure 3. Antibiotic resistance rates of ESBL-producing *E. coli* isolates.
All ESBL-producing isolates (100%) were multi-antibiotic resistant.

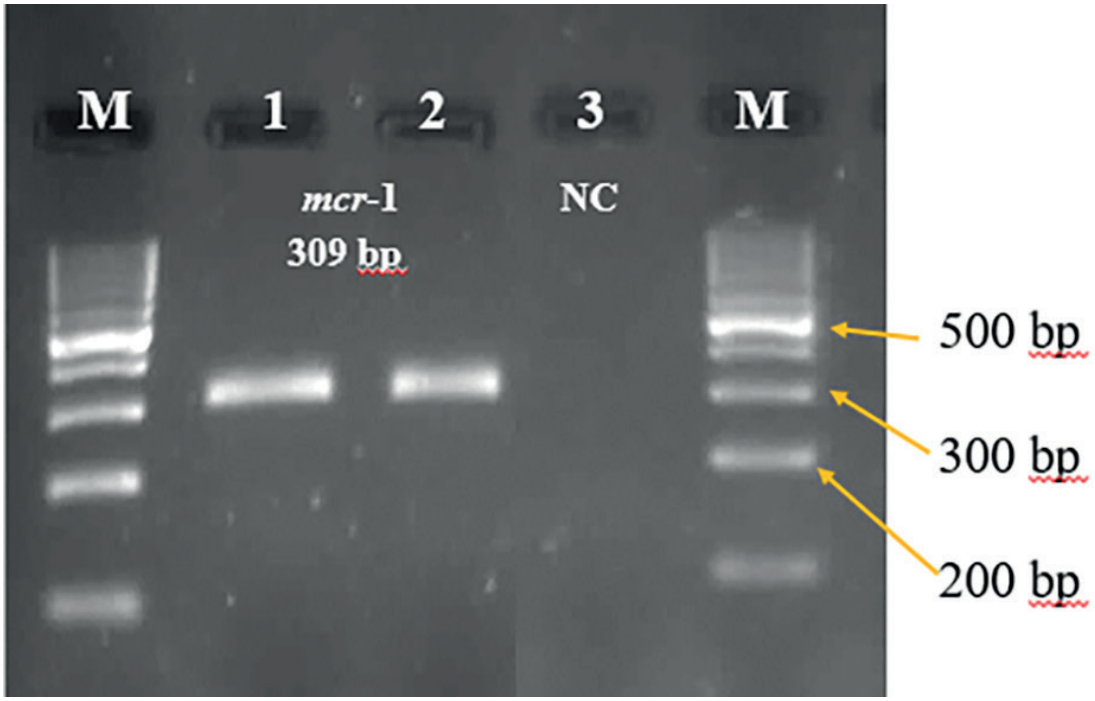

Figure 4. Gel electrophoresis for colistin resistance encoded by the *mcr*-1 gene: **1.** *mcr*-1 gene (309 bp, *E. coli* NCTC 13846); **2.** *mcr*-1 gene positive field isolate; **3.** NC (*E. coli* ATCC 25922); **M:** Marker (100 bp, Fermentas, USA).

producing and multidrug resistant *E. coli* strains isolated from broilers in western Türkiye were investigated. The findings indicate a possible zoonotic transmission risk and that these bacteria limit treatment options. In particular, the coexistence of two critically important resistance mechanisms, such as ESBL and colistin resistance, reduces the ability of bacteria to respond to treatment, posing a serious risk for both veterinary and public health.

In recent years, the prevalence of ESBL-producing *E. coli* strains has been increasing worldwide and this is a significant problem, especially in poultry farming (5). It has been reported that ESBL production is common in *E. coli* strains isolated from poultry in Brazil and that these strains have developed resistance to a wide range of antibiotics (33). Similarly, a high prevalence of ESBL-producing strains was detected in our study, and it was observed that the majority

of these strains were resistant to broad-spectrum antibiotics. This confirms once again that the use of antibiotics in the livestock industry contributes to the development of resistance in bacteria (34). In particular, the widespread use of broad-spectrum antibiotics in animal farming accelerates the spread of antibiotic resistance.

The potential for transmission of ESBL-producing bacteria from animals to humans poses a serious threat to public health. A previous study emphasized that transmission of ESBL-producing *E. coli* strains commonly found in poultry has become a global problem (35). Our findings are consistent with this global trend and indicate that the poultry production chain plays a critical role in the spread of antimicrobial resistance (36). This situation highlights the need to develop effective strategies to ensure the safety of animal products and combat antimicrobial resistance.

In our study, the most common ESBL genotype was the *bla*$_{\text{TEM}}$ gene, with a prevalence of 53%; this prevalence is higher than the prevalence in a study conducted in Brazil (17%) (33) and lower than the prevalence in Egypt (100%) (37). The second most common ESBL genotype was the *bla*$_{\text{CTX-M}}$ gene, with a prevalence of 27%. This prevalence is higher than some studies in South America (38), but lower than other studies (39). In addition, the *bla*$_{\text{SHV}}$ gene was detected with a low prevalence of 8% in our study, whereas the prevalence of this gene in poultry farms in Brazil was reported as high as 45% (33). In Germany and Spain, *bla*$_{\text{SHV}}$-like enzymes have been reported to be common in retail poultry products (40). The prevalence of the rarely studied *bla*$_{\text{OXA}}$ gene was 5% in our study, while it was reported as 11% in the Egyptian study (37). These proportional differences may be due to various factors such as geographical variations and antibiotic utilization policies.

The antibiotic susceptibility results obtained in our study are parallel to the findings of a similar study conducted in Algeria (18). High resistance rates to antibiotics such as ampicillin (100%), tetracycline (100%), nalidixic acid (95%) and ciprofloxacin (87%) have been reported in avian pathogenic *E. coli* strains isolated in Algeria. Similarly, high resistance rates to these antibiotics were observed in our study. However, trimethoprim-sulfamethoxazole resistance was reported at a lower rate of 62% in the Algerian study, while it was 82% in our study. These findings are consistent with the study conducted in Algeria in 2023 (18) and emphasize the high prevalence of multiple antibiotic resistant strains. Differences in antibiotic resistance rates may be due to geographical, socio-economic factors and diversity in antibiotic use habits.

The risk of transmission of the *mcr*-1 gene from animals to humans through direct contact, contaminated food, and environmental sources is high (41). The spread of this gene threatens public health by causing infections that limit treatment options in humans and nosocomial infections (42). In our study, the mcr-1 gene was detected in 12% of ESBL-producing isolates; this finding is consistent with previous studies. In a study conducted in Argentina in 2019, the presence of the *mcr*-1 gene was confirmed in *E. coli* strains isolated from domestic animals and it was stated that this gene plays a critical role in the spread of resistance between humans and animals (38). The ability of the *mcr*-1 gene to be transmitted via plasmid allows rapid spread of resistance and poses a risk especially between poultry and domestic animals (42). Colistin is one of the antibiotics of last resort, and the presence of *mcr*-1-carrying strains severely limits treatment options (30, 41). The low rates of colistin resistance observed in our study indicate that careful management of antibiotics may be effective in limiting the development of resistance, but other environmental and genetic factors also play a role. In a recent study conducted in Algeria (18), 14% colistin resistance was reported, in line with the findings in this study. The rapid spread of resistance genes via horizontal gene transfer (conjugation, transduction, transformation) via plasmids is accelerated in environments with high antibiotic pressure, especially via conjugation. In environments where antibiotics are used intensively, the mobilization ability of plasmids and environmental stress factors promote this gene transfer (30). This situation may cause the rapid spread of resistant bacteria, especially in places such as hospitals and animal husbandry facilities.

Differences in antibiotic use among countries have a significant impact on the prevalence of ESBL-producing bacteria. While antibiotic use is strictly regulated in developed countries, uncontrolled and widespread antibiotic use is still a major problem in developing countries. For example, the ban on growth-promoting antibiotics in the European Union has reduced the spread of ESBL strains (43), while in countries such as India and Brazil, the easy accessibility of antibiotics and their uncontrolled use in animal husbandry have led to an increase in antibiotic resistance (43). Similarly, in countries such as the USA and China, the use of antibiotics in the animal husbandry sector contributes to the spread of resistant

bacteria (42, 44). Similarly, in Türkiye, the uncontrolled use of antibiotics in animal husbandry, especially due to over-the-counter sales and inadequate regulations, contributes to the increase in antibiotic resistance. This situation highlights the need for stricter regulations at the local and global level to limit antibiotic resistance.

Our study demonstrates the presence of both ESBL production and the *mcr*-1 gene in *E. coli* strains isolated from broilers in Türkiye, indicating that these two important resistance mechanisms coexist. In particular, the dominance of the *bla*$_{\text{TEM}}$ genotype, the increasing prevalence of the *bla*$_{\text{CTX-M}}$ gene, and the detection of the *mcr*-1 gene at a rate of 12% indicate regional differences in the antimicrobial resistance profile in poultry farming in Türkiye. These findings, while being consistent with the data reported in the international literature, suggest that Türkiye's different geographical and production conditions make significant contributions to the dynamics of resistance spread. The increase in multiple antibiotic resistance and colistin resistance causes a decrease in the effectiveness of existing antibiotics, necessitating the development of new treatment strategies. These strategies include innovative approaches such as new antibiotic classes, bacteriophage treatments, and antimicrobial peptides. In addition, methods that reduce the risk of resistance, such as probiotics, can be evaluated among alternative treatment options. Given that multiple antibiotic resistance and colistin resistance limit current treatment options, the development and implementation of such alternatives is of critical importance.

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REFERENCES

1. Paterson, D.L. and Bonomo, R.A.: Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. 18(4):657-86, 2005.
2. Amato, H.K., Loayza, F., Salinas, L., Paredes, D., Garcia, D., Sarzosa, S., Saraiva-Garcia, C., Johnson, T.J., Pickering, A.J., Riley, L.W., Trueba, G. and Graham, J.P.: Risk factors for extended-spectrum beta-lactamase (ESBL)-producing *E. coli* carriage among children in a food animal-producing region of Ecuador: A repeated measures observational study. PLos Med., 13;20(10):e1004299, 2023.
3. Cao, H., Bougouffa, S., Park, T., Lau, A., Tong, M., Chow, K. and Ho, P.: Sharing of antimicrobial resistance genes between humans and food animals. mSystems7:e00775-22, 2022.
4. Bubpamala, J., Khuntayaporn, P., Thirapanmethee, K., Montakantikul, P., Santanirand, P. and Chomnawang, M.T.: Phenotypic and genotypic characterizations of extended-spectrum beta-lactamase-producing *Escherichia coli* in Thailand. Infect. Drug. Resist. 5;11:2151-2157, 2018.
5. Castanheira, M., Simner, P.J. and Bradford, P.A.: Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob. Resist. 16;3(3):dlab092, 2021.
6. Hussain, H.I., Aqib, A.I., Seleem, M.N., Shabbir, M.A., Hao, H., Iqbal, Z., Kulyar, M.F., Zaheer, T. and Li, K.: Genetic basis of molecular mechanisms in β-lactam resistant Gram-negative bacteria. Microb. Pathog. 158:105040, 2021.
7. Cantón, R., González-Alba, J.M. and Galán, J.C.: CTX-M enzymes: origin and diffusion. Front Microbiol. 2;3:110, 2012.
8. Liakopoulos, A. Mevius, D. and Ceccarelli, D.: A review of SHV extended-spectrum b-lactamases: neglected yet ubiquitous. Front. Microbiol. 7:1374, 2016.
9. Evans, B.A. and Amyes, S.G.: OXA β-lactamases. Clin. Microbio.l Rev. 27(2):241-63, 2014.
10. Yoon, S. and Lee, Y.J.: Molecular characteristics of ESBL-producing *Escherichia coli* isolated from chickens with colibacillosis. J. Vet. Sci. May;23(3):e37, 2022.
11. Falgenhauer, L., Imirzalioglu, C., Oppong, K., Akenten, C.W., Hogan, B., Krumkamp, R., Poppert, S., Levermann, V., Schwengers, O. and Sarpong, N.: Detection and characterization of ESBL-producing *Escherichia coli* from humans and poultry in Ghana. Front. Microbiol. 9, 3358, 2019.
12. Chishimba, K., Hang'Ombe, B., Muzandu, K., Mshana, S., Matee, M., Nakajima, C. and Suzuki, Y.: Detection of extended-spectrum beta-lactamase-producing *Escherichia coli* in market-ready chickens in Zambia. Int. J. Microbiol. 5275724, 2016.
13. Guenther, S., Aschenbrenner, K., Stamm, I., Bethe, A., Semmler, T., Stubbe, A., Stubbe, M., Batsajkhan, N., Glupczynski, Y. and Wieler, L.H.: Comparable high rates of extended-spectrum-beta-lactamase-producing *Escherichia coli* in birds of prey from Germany and Mongolia. PLoS ONE 7, e53039, 2012.
14. Badr, H., Reda, R.M., Hagag, N.M., Kamel, E., Elnomrosy, S.M., Mansour, A.I., Shahein, M.A., Ali, S.F. and Ali, H.R.: Multidrug-resistant and genetic characterization of extended-spectrum beta lactamase producing *E. coli* recovered from chickens and humans in Egypt. Animals. 12: 346, 2022.
15. Ünal, N., Karagöz, A., Aşkar, Ş., Dilik, Z. and Yurtyeri, B.: Extended-spectrum β-lactamases among cloacal *Escherichia coli* isolates in healthy broilers in Türkiye. Turk. J. Vet. Anim. Sci. 41(1): 72-76, 2017.
16. Çil, G.İ., Cengiz, G., Aslan, B. and Şireli, U.F.: Determination of extended spectrum beta-lactamase producing *Escherichia coli* strains in chicken meat samples. Eurasian J. Vet. Sci. 36, 3, 187-192, 2020.
17. Aslantaş, Ö. and Gövce, N.: Isolation and molecular characterization of ESBL/pAmpC producing *Escherichia coli* in household pigeons in Türkiye. Isr. J. Vet. Med. 73(4):23-29, 2018.
18. Aberkane, C., Messaï, A., Messaï, C.R. and Boussaada, T.: Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. Vet. World. 16(3):449-454, 2023.

19. Seferoglu, Y., Turkyilmaz, M.K. and Turkyilmaz, S.: Investigation of antibiotic resistance, phylogenetic groups and clonal relationships of colistin resistant *Escherichia coli* isolates obtained from broilers. Isr. J. Vet. Med. 79(1): 27-40, 2024.
20. Cochran, W. G. (1977). Sampling Techniques. 3rd Edition. Wiley.
21. Koneman E, Stephen A, Gary P. Koneman's color atlas and textbook of diagnostic microbiology. Philadelphia: Lippincott Williams and Wilkins; 2017. p. 360-6.
22. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing Standards*. 30th ed., CLSI supplement M100. Clinical and Laboratory Standards Institute; 2020.
23. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters, version 12.0; http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf. Accessed August 18, 2022.
24. Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbart, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L.: Multidrug-resistant, extensively drug resistant and pandrug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268-281, 2012.
25. Turner, P., Mclennan, A., Bates, A. and White, M.: BIOS Instant Notes in Molecular Biology. 4th Edition, Taylor & Francis, England, 2012.
26. Chen, J. and Griffiths, M.W.: PCR differentiation of *Escherichia coli* from other gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. Lett. Appl. Microbiol. 27(6):369-71, 1998.
27. Bali, E.B., Acık, L. and Sultan, N.: Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum beta-lactamase produced by *Escherichia coli*, *Acinobacter baumannii* and *Klebsiella* isolates in a Turkish hospital. Afr. J. Microbiol. Res. 4(8):650-4, 2010.
28. Weill, F.X., Demartin, M., Tande, D., Espie, E., Rakotoarivony, I. and Grimont, P.A.D.: SHV-12-like extended-spectrum- -lactamase-producing strains of salmonella enterica serotypes babelsberg and enteritidis isolated in France among infants adopted from Mali. J. Clinical Microbiol. 42(6):2432-7, 2004.
29. Gallardo, F., Marco, F., Vila, J., Ruiz, J. and Towner, K.J.: Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of Salmonella serotype Typhimurium with investigation of molecular epidemiology and mechanisms of resistance. J. Med. Microbiol. 48(4): 367-74, 1999.
30. Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R. and Spencer, J.: Emergence of plasmid-mediated colistin resistance mechanism *mcr*-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infect. Dis. 16(2), 161-168, 2016.
31. Poirel, L., Madec, J.-Y., Lupo, A., Schink, A.K., Kieffer, N., Nordmann, P. and Schwarz, S.: Antimicrobial resistance in *Escherichia coli*. Microbiol. Spectr. 6(4), 2018.
32. Cardozo, M.V., Liakopoulos, A., Brouwer, M., Kant, A., Pizauro, L.J.L., Borzi, M.M., Mevius, D. and de Ávila, F.A.: Occurrence and molecular characteristics of extended-spectrum betalactamase-producing enterobacterales recovered from chicken, chicken meat, and human infections in Sao Paulo State, Brazil. Front. Microbiol. 12, 628738, 2021.
33. Patricio, T. C. C., Farias, B. O., Santiago, G. S., Souza, V. R. S., Pimenta, R. L., Oliveira, C. C., Coelho, I. S., Souza, M. M. S. and Coelho, S. M. O.: Production of extended-spectrum beta-lactamases in *Escherichia coli* isolated from poultry in Rio de Janeiro, Brazil. Braz. J. Vet. Med., 44, e001722, 2022.
34. Marshall, B. M. and Levy, S. B.: Food animals and antimicrobials: Impacts on human health. Clin. Microbiol. Rev., 24(4), 718-733, 2011.
35. Plaza Rodríguez, C., Correia Carreira, G. and Käsbohrer, A.: A probabilistic transmission model for the spread of extended-spectrum-β-lactamase and AmpC-β-lactamase-producing *Escherichia coli* in the broiler production chain. Risk Anal., 38(12), 2659-2682, 2018.
36. Brower, C. H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S. J., Kaur, R., Chatterjee, L., Mishra, S., Das, B. R., Singh, P., Singh, R., Gill, J. P. S. and Laxminarayan, R.: The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environ. Health Perspect., 125(7), 077015, 2017.
37. Saad, D.N., Sultan, S., Abdelhalem, M.A., Abdul Azeem, M. W. A.: Molecular detection of *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{OXA}}$ from *Escherichia coli* isolated from chickens. J. Vet. Ani. Res. 2:102, 2019.
38. Rumi, M. V., Mas, J., Elena, A., Cerdeira, L., Muñoz, M. E., Lincopan, N., Gentilini, É. R., Di Conza, J. and Gutkind, G.: Co-occurrence of clinically relevant β-lactamases and MCR-1 encoding genes in *Escherichia coli* from companion animals in Argentina. Vet. Microbiol., 230, 228-234, 2019.
39. Tong, P., Sun, Y., Ji, X., Du, X., Guo, X., Liu, J., Zhu, L., Zhou, B., Zhou, W., Liu, G. and Feng, S.: Characterization of antimicrobial resistance and extended-spectrum β-lactamase genes in *Escherichia coli* isolated from chickens. Foodborne Pathog. Dis., 12(4), 345-352, 2015.
40. Alonso, C. A., Michael, G. B., Li, J., Somalo, S., Simón, C., Wang, Y., Kaspar, H., Kadlec, K., Torres, C. and Schwarz, S.: Analysis of *bla*SHV-12-carrying *Escherichia coli* clones and plasmids from human, animal and food sources. The Journal of Antimicrobial Chemotherapy, 72(6), 1589-1596, 2017.
41. Roth, N., Kasbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C. and Domig, K. J.: The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. Poult. Sci., 98(4), 1791-1804, 2019.
42. Wu, C., Wang, Y., Shi, X., Wang, S., Ren, H., Shen, Z., Wang, Y., Lin, J. and Wang, S.: Rapid rise of the ESBL and *mcr*-1 genes in *Escherichia coli* of chicken origin in China, 2008-2014. Emerg. Micro. Infect., *7*(1), 30, 2018.
43. Ribeiro, L.F., Nespolo, N.M., Rossi, G.A.M. and Fairbrother, J.M.: Exploring extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in food-producing animals and animal-derived foods. Pathogens. 22;13(4):346, 2024.
44. Rabello, R.F., Bonelli, R.R., Penna, B.A., Albuquerque, J.P., Souza, R.M. and Cerqueira, A.M.F.: Antimicrobial resistance in farm animals in Brazil: An update overview. Animals, 10, 552, 2020.