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Research Article

Investigation of Mobilized Colistin Resistance Gene-1 in Poultry Pathogenic *Escherichia coli*

Ecenur YILMAZ[1], Süheyla TÜRKYILMAZ[2*]

[1]*Aydın Adnan Menderes University, Health Sciences Institute, Aydın, TÜRKİYE*

[2]*Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Aydın, TÜRKİYE*

ABSTRACT

This study aimed to determine the presence of the mobilized colistin resistance gene 1 (*mcr*-1), which provides plasmid-mediated colistin resistance in avian pathogenic *Escherichia coli* (APEC) isolates, and to examine the antibiotic resistance profiles of colistin resistant isolates. In this study, isolates from 200 broilers with suspicion of colibacillosis from previous studies were used as material. Following the isolation of *E. coli* through classical conventional methods, identification and antibiotic susceptibility tests were conducted using an automated microbiology system (BD Phoenix 100TM, USA). The presence of the *mcr*-1 gene in isolates phenotypically determined as colistin resistant was investigated using polymerase chain reaction (PCR). Ten (6.4%) out of one hundred fifty-six *E. coli* isolates were found to be phenotypically resistant to colistin. It was found that 80% of colistin resistant *E. coli* isolates were resistant to l evofloxacin, 70% to cefazolin and cefuroxime, 60% to ceftazidime, 50% to gentamicin and ceftriaxone, 40% to cefepime, 30% to ceftolozane-tazobactam, and 20% to piperacillin-tazobactam. All isolates were sensitive to amikacin, ertapenem, imipenem, meropenem, and resistant to ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and tigecycline, exhibiting multidrug resistance (MDR). All isolates that were found to be phenotypically resistant to colistin also carried the *mcr*-1 gene. These findings indicate that *mcr* in APEC contributes to the rapid spread of plasmid-mediated resistance genes and the escalation of broad-spectrum antibiotic resistance. The detection of resistance to antibiotics used in human medicine may pose a potential threat to public health. Future studies should be conducted with samples from different regions and include a diverse sample group to better understand this risk.

Keywords: *Antibiotic resistance, avian pathogenic Escherichia coli, mobile colistin resistance gene.*

Kanatlı Patojenik *Escherichia coli*'lerde Mobilize Kolistin Direnç Geni-1'in Araştırılması

ÖZET

Bu çalışma, kanatlı patojenik *Escherichia coli* (APEC) izolatlarında plazmit aracılı kolistin direnci sağlayan mobilize kolistin direnç geni 1 (*mcr*-1) varlığının belirlenmesi ve kolistin dirençli izolatların antibiyotik direnç profillerinin incelenmesi amaçlandı. Çalışmada, kolibasillozis şüpheli 200 broylerden daha önceki çalışmalar kapsamında elde edilen izolatlar materyal olarak kullanıldı. *E. coli* izolasyonları klasik konvansiyonel yöntemler ile gerçekleştirildikten sonra identifikasyonlar ve antibiyotik duyarlılık testleri otomatize mikrobiyoloji sistemi (BD Phoenix 100™, ABD) sistemi yardımı ile yapıldı. Fenotipik olarak kolistin dirençli olduğu tespit edilen izolatlarda *mcr*-1 gen varlığı polimeraz zincir reaksiyonu (PZR) ile incelendi. Yüz elli altı *E. coli*'nin 10 (%6,4)'unun fenotipik olarak kolistin dirençli olduğu saptandı. Kolistin dirençli *E. coli* izolatların %80'inin levofloksasine, %70'inin sefazolin ve sefuroksime, %60'ının sefotazidime, %50'sinin gentamisin ve seftriaksona, %40'ının sefepime, %30'unun seftolozan tazobaktama, %20'sinin piperasilin tazobaktama dirençli oldukları belirlendi. Tüm izolatlar amikasin, ertapenem, imipenem, meropenem duyarlı; ampisilin, amoksisilin klavulanat, ampisilin sulbaktam, trimethoprim sulfamethoksazol ve tigesiklin dirençli olup; çoklu antibiyotik direncine (MDR) sahip idi. Fenotipik olarak kolistin dirençli oldukları tespit edilen tüm izolatların genotipik olarak da *mcr*-1 genini taşıdıkları saptandı. Bulgular, APEC izolatlarında kolistin direncinin mobilize olmasının, plazmidik direnç genlerinin hızlı yayılmasına ve antibiyotik direncinin artmasına katkı sağladığını göstermektedir. Beşeri hekimlikte de kullanılan antibiyotiklere direnç saptanması halk sağlığı için tehdit oluşturabilir. Gelecekteki çalışmalar, farklı bölgelerden alınan örneklerle yapılmalı ve bu riskin daha iyi anlaşılması için geniş bir örneklem grubunu içermelidir.

Anahtar kelimeler: *Antibiyotik direnci, kanatlı patojenik Escherichia coli, mobilize kolistin direnç geni.*

***Corresponding author:** Süheyla TÜRKYILMAZ, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Aydın, TÜRKİYE. sturkyilmaz@adu.edu.tr*

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Introduction

In recent years, antimicrobial resistance against bacteria poses a serious threat to global public health. The increasing use of antibiotics in both animals and humans, particularly in the case of animals, leads to the spread of antimicrobial resistance in society (Salam et al., 2023). The use of antimicrobials in animals for disease control or growth promotion results in commensal microflora acquiring resistance genes from resistant strains, with horizontal gene transfer being the underlying mechanism of this process (Salinas et al., 2019). Studies indicate that antimicrobial resistance in humans can arise through horizontal transfer of antibiotic resistance genes from foodborne sources or direct transfer of resistant bacteria (Marshall et al., 2011).

Escherichia coli, a microorganism belonging to the *Enterobacteriaceae* family, is found in the intestines of both animals and humans. However, it can cause life threatening infections in animals and humans, especially in poultry. Antimicrobial resistant *E. coli* strains pose a potential risk to public health and can function as carriers capable of transferring antimicrobial resistance determinants to their own strains or other bacterial species (Rasheed et al., 2014).

Colistin, previously avoided in human medicine due to systemic toxicity, has been reintroduced for the treatment of Gram-negative bacteria showing multidrug resistance (MDR). Colistin is critically important as a last-resort antibiotic in human medicine (Poirel et al., 2017).

Colistin is also used in veterinary medicine for disease control and growth promotion in food-producing animals (Kempf et al., 2016; Apostolakos & Piccirillo, 2018). The widespread use of colistin in animals can lead to the emergence of colistin resistance in animal-origin bacteria and its transmission to humans (Poirel and Nordmann, 2016). The origin of resistant microorganisms in humans is thought to be cattle and food-producing animals as chickens. Until the discovery of the transferable plasmid-mediated *mcr*-1 gene in 2015, colistin resistance in bacteria was considered to result from chromosomal mutations. The emergence of mcr-mediated colistin resistance poses a significant threat to the treatment of infections (Liu et al., 2016).

Since the initial report of *mcr*-1, many studies have continuously reported new *mcr* genes in *E. coli* worldwide (Lemlem et al., 2023). Following the discovery of *mcr*-1 in China, numerous studies on colistin resistance, especially in food animals including poultry, have been conducted globally, particularly in Asia (Kempf et al., 2016). Many countries have banned the use of colistin as a growth promoter in food additives due to the increased in colistin resistance in animals (Wang et al., 2020). Although colistin resistance has decreased after the complete ban on colistin use in animal production, significant levels of colistin resistance are still reported worldwide, especially in pigs and poultry (Wang et al., 2020). Commonly reported genes encoding colistin resistance include those from *mcr*-1 to *mcr*-10 (Valiakos et al., 2021).

While studies on colistin resistance in Türkiye are limited (Kurekci et al., 2018; Adıgüzel et al., 2021; Erzaim and İkiz, 2021; Aslantaş and Küçükaltay, 2023; Seferoglu et al., 2024). An *mcr*-1 positive *E. coli* isolate reported in Hatay stands out as the first case in the country (Kurekci et al., 2018). Studies conducted in Erzurum and İstanbul also indicate the persistence of colistin resistance in isolates obtained from chicken meat, highlighting it as a significant problem across a wide geography (Adıgüzel et al., 2021; Erzaim and İkiz, 2021).

As of our current knowledge, there is no information regarding the presence of *mcr*-1 mediating colistin resistance in APEC isolates in Aydın province in western Türkiye, and there is no information about the antibiotic resistance profiles of colistin-resistant isolates. This study was conducted to determine the presence of the *mcr*-1 gene in APEC isolates and to examine the antibiotic resistance profiles of colistin-resistant isolates. This study focuses on an important issue for both veterinary medicine and public health, serving as a fundamental in the development of broader and more effective strategies to combat colistin resistance. Additionally, the results of the antibiotic resistance testing will guide veterinarians in directing treatment strategies and monitoring antimicrobial resistance.

Materials and Methods

Ethical Approval

Isolates obtained from previous studies, brought to Aydın Adnan Menderes University Faculty of Veterinary Medicine, Department of Microbiology, Routine Diagnostic Laboratory for routine diagnosis purposes, were used in the study.

Animal Material

In this study, isolates obtained from 200 chickens brought to the Laboratory of Microbiology Department, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, for disease diagnosis throughout the year 2023 (January-December) were used, based on previous studies.

Bacterial Isolation and Identification

Isolates obtained in previous studies were revitalized and purity checks were performed. Isolates were plated on MacConkey agar (Merck 105465, Germany) and incubated aerobically at 37 °C for 24 hours. The following day, a single lactose-positive colony on MacConkey agar was subcultured onto EMB agar (Merck 101347, Germany). After another 24 hours of incubation at 37 °C, *E. coli* colonies exhibiting a characteristic green metallic sheen were selected. These colonies underwent biochemical tests (motility, oxidase, catalase, indole, etc.) (Koneman et al., 1997). Bacterial identification was confirmed using an automated system (BD Phoenix, Becton-Dickinson, USA) following the manufacturer's instructions. Isolates were stored in Brain Heart Infusion Broth (BHIB) supplemented with 20% glycerol (Merck 110493, Germany) at

-20°C.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing (AST) of the *E. coli* isolates was conducted utilizing the BD Phoenix 100™ automated system (Becton-Dickinson, USA) with NMIC/ID 433 panels. A comprehensive panel of 20 antibiotics, spanning nine antimicrobial families, was employed for testing, including aminoglycosides (amikacin, gentamicin), carbapenems (ertapenem, imipenem, meropenem), cephems (cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime), penicillin (ampicillin), beta-lactams (ceftolozane-tazobactam, amoxicillin clavulanate, ampicillin sulbactam, piperacillin-tazobactam), lipopeptide (colistin), folate (trimethoprim-sulfamethoxazole), quinolones (ciprofloxacin, levofloxacin), and tetracycline (tigecycline). The resistance profiles of the isolates to these antibiotics were determined, with interpretation based on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2022). *E. coli* ATCC 25922 strains were used for quality control. Additionally, colistin resistance was specifically assessed in all isolates using the automated system, and the minimum inhibitory concentration (MIC) results were interpreted according to EUCAST clinical breakpoints (susceptible ≤ 2 mg/l; resistant > 2 mg/l) (EUCAST, 2022).

Multidrug Resistance (MDR)

MDR was characterised as resistance to three or more classes of antimicrobial agents (Magiorakos et al., 2012).

Polymerase Chain Reaction

DNA Extraction, Purity, and Quantification Controls

In this study, DNA was extracted using the sonication method (Maniatis & Sambrook, 1989). *E. coli* stock cultures were plated on EMB agar and incubated at 37 °C for 24 hours. A single colony was selected and transferred to 5 ml of nutrient BHIB broth and incubated at 37 °C for 18-24 hours. After centrifugation at 13,500 rpm for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 200 µl of PBS (~10^8/ml). The suspension was sonicated at 40 Hz for 10 minutes and then centrifuged at 13,500 rpm for 5 minutes. The resulting supernatant was collected, and DNA concentration was assessed using a nanodrop (Thermo Fisher Scientific, Waltham, MA, USA) to ensure purity and quantify DNA (Turner et al., 2012). Samples with OD260/280 values between 1.6 and 2.0 were considered sufficiently pure. Finally, 3 µl of template DNA was used in each PCR reaction.

Genotypic Detection of Colistin Resistance

Primers previously designed by Lui et al. were used to amplify the *mcr*-1 gene (Liu et al., 2016). The *mcr*-1 gene was detected by conventional PCR in ten isolates that were phenotypically resistant to colistin. The product size of the *mcr*-1-positive amplicon was 309 bp. Bands of the expected size were visualised on a 2% agarose gel after electrophoresis at 100 V for 45 min. The genomic DNA of the *E. coli* NCTC 13846 strain was used as a positive control and the *E. coli* ATCC 25922 strain was used as a negative control in the polymerase chain reactions.

Results

Bacterial Isolation and Identification

In this study, 156 (78.8%) *E. coli* isolates were obtained from 200 broilers suspected of colibacillosis. Gram-negative rod-shaped isolates with a metallic green sheen on EMB agar and forming pink colonies on MacConkey agar, along with negative oxidase and positive catalase and indole tests, were considered suspicious for *E. coli*.

All 156 suspected *E. coli* isolates were identified as *E. coli* using NMIC/ID 433 panels in the BD Phoenix 100™ automated microbiology system. The biochemical test results of the isolates as a result of the identification process carried out with the help of the automated testing system are shown in Table 1.

Antibiotic Susceptibility Testing

An automated microbiology system was used to determine the resistance status of 156 *E. coli* isolates to 20 antimicrobial drugs belonging to nine antimicrobial families. As a result of the antibiotic susceptibility test, 10 (6.4%) of the 156 *E. coli* isolates were found to be phenotypically resistant to colistin. The results of the antibiotic susceptibility testing of ten colistin-resistant isolates are shown in Table 2, Table 3 and Figure 1.

Eight (80%) of the colistin-resistant *E. coli* isolates were found to be resistant to levofloxacin; 7 (70%) to cefazolin and cefuroxime; 6 (60%) to ceftazidime; 5 (50%) to gentamicin and ceftriaxone; 4 (40%) to cefepime; 3 (30%) to ceftolozane tazobactam; 2 (20%) to piperacillin tazobactam. While all colistin-resistant isolates were sensitive to amikacin, ertapenem, imipenem, and meropenem; it was resistant to ampicillin, amoxicillin clavulanate, ampicillin sulbactam, trimethoprim sulfamethoxazole and tigecycline.

While it was determined that *E. coli* isolates showed low rates (10%-30%) of resistance to some antibiotics (ceftolozane-tazobactam, piperacillin-tazobactam), moderate rates (31%-75%) of resistance to many antimicrobials (gentamicin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime); and high levels of resistance (80%-100%) to others (ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, colistin, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline). While the most effective antibiotics against isolates were amikacin, ertapenem, imipenem, meropenem; ampicillin, amoxicillin clavulanate, ampicillin-sulbactam, trimethoprim-sulfamethoxazole and tigecycline were ineffective antibiotics.

The number of antibiotics to which colistin-resistant *E. coli* isolates are resistant, moderately sensitive and sensitive according to the antibiotic susceptibility test is shown in Table 4.

While one isolate 7, one isolate 8, two isolates 9, two isolates 11, two isolates 12, two isolates 13 were resistant to antibiotics; three isolates were resistant to 6, two isolates 7, one isolate 8, two isolates 9, one isolate 10, and one isolate was susceptible to 12 antibiotics.

Multiple Antibiotic Resistance

All ten (100%) colistin-resistant *E. coli* isolates obtained from broilers with colibacillosis had multiple antibiotic resistance.

Of the ten colistin-resistant *E. coli* isolates, 7 (70%) were resistant to 6 and 3 (30%) were resistant to 7 antimicrobial families.

Table 1. Biochemical test results of *E. coli* isolates

Test	Result	Test	Result	Test	Result
A-ARARR	V	A-GLPRB	-	A-GLYB	-
A-GUGAH	-	A-LARGH	-	A-LGTA	V
A-LEUH	V	A-LPHET	-	A-LPROB	-
A-LPYR	-	A-LTRY	-	A-LYALD	V
A-ACT	-	C-ADO	-	C-CIT	-
C-CLST	V	C-DMNT	V	C-KGA	V
C-MLO	-	C-PXB	V	C-TIG	-
M-NAG	-	N-LGGH	-	N-LPROT	V
P-BDGLU	-	P-BPHO	V	R-BALL	V
R-BGEN	-	R-DEX	+	R-DFRU	+
R-DGAL	V	R-DGUA	+	R-DMLB	V
R-DSBT	V	R-DSUC	-	R-GRA	+
R-LARA	+	R-LRHA	V	R-MBGU	-
R-MTU	-	R-NGA	V	R-NGU	+
S-ORN	V	S-URE	-	t-ESC	-

V: Variable

Table 2. Antibiotic susceptibility test results of colistin-resistant isolates

Antimicrobial Family	Antibiotic Name	1	2	3	4	5	6	7	8	9	10
Aminoglycoside	Amikacin	S	S	S	S	S	S	S	S	S	S
	Gentamicin	S	R	S	R	R	S	S	S	R	R
Carbapenem	Ertapenem	S	S	S	S	S	S	S	S	S	S
	Imipenem	S	S	S	S	S	S	S	S	S	S
	Meropenem	S	S	S	S	S	S	S	S	S	S
Cephem	Cefazolin	R	R	R	R	I	R	R	I	R	S
	Cefuroxime	R	R	I	R	S	R	R	R	R	S
	Ceftazidime	R	R	S	S	S	R	R	R	R	S
	Ceftriaxone	R	R	S	S	S	S	R	R	R	S
	Cefepime	R	R	S	S	S	S	S	R	R	S
Penicillin	Ampicillin	R	R	R	R	R	R	R	R	R	R
Beta lactam	Ceftolozane tazobactam	S	S	S	S	S	R	R	R	S	S
	Amoxicillin clavulanate	R	R	R	R	R	R	R	R	R	R
	Ampicillin sulbactam	R	R	R	R	R	R	R	R	R	R
	Piperacillin tazobactam	S	S	R	S	S	S	S	S	S	S
Folate	Trimethoprim sulfamethoxazole	R	R	R	R	R	R	R	R	R	R
Quinolone	Ciprofloxacin	R	R	R	R	R	R	R	R	R	R
	Levofloxacin	R	R	R	R	R	R	I	R	R	S
Tetracycline	Tigecycline	R	R	R	R	R	R	R	R	R	R

S: Susceptible, I: Intermediate, R: Resistant

Polymerase Chain Reaction

DNAs obtained from all isolates were examined molecularly by polymerase chain reaction for the presence of the *mcr*-1 gene. All ten isolates that were found to be phenotypically resistant to colistin were also found to carry the *mcr*-1 gene genotypically (Figure 2).

All phenotypically colistin-resistant isolates had the plasmid-mediated *mcr*-1 gene.

Discussion

Colistin, also known as polymyxin E, is a decapeptide antimicrobial compound discovered shortly after World War II. However, its use has been restricted due to concerns about systemic toxicity which poses a serious risk to public health. In recent years, there has been an increased reutilization of colistin as a last-resort antibiotic against infections caused by multidrug-resistant Gram-negative

Table 3. Susceptibility and resistance status of colistin-resistant *E. coli* isolates to antibiotics

Antibiotic Name	Total (n=10)		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Amikacin	0 (0)	0 (0)	10 (100)
Gentamicin	5 (50)	0 (0)	5 (50)
Ertapenem	0 (0)	0 (0)	10 (100)
Imipenem	0 (0)	0 (0)	10 (100)
Meropenem	0 (0)	0 (0)	10 (100)
Cefazolin	7 (70)	2 (20)	1 (10)
Cefuroxime	7 (70)	1 (10)	2 (20)
Ceftazidime	6 (60)	0 (0)	4 (40)
Ceftriaxone	5 (50)	0 (0)	5 (50)
Cefepime	4 (40)	0 (0)	6 (60)
Ampicillin	10 (100)	0 (0)	0 (0)
Ceftolozane tazobactam	3 (30)	0 (0)	7 (70)
Amoxicillin clavulanate	10 (100)	0 (0)	0 (0)
Ampicillin sulbactam	10 (100)	0 (0)	0 (0)
Piperacillin tazobactam	2 (20)	0 (0)	8 (80)
Trimethoprim sulfamethoxazole	10 (100)	0 (0)	0 (0)
Ciprofloxacin	10 (100)	0 (0)	0 (0)
Levofloxacin	8 (80)	1 (10)	1 (10)
Tigecycline	10 (100)	0 (0)	0 (0)

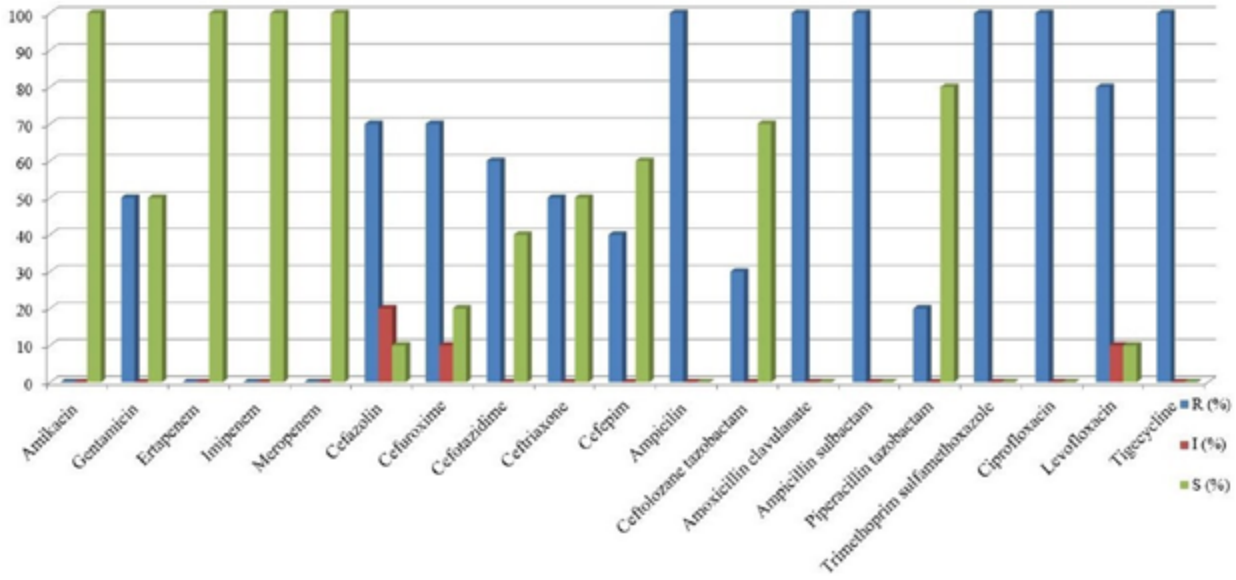

Figure 1. Resistance and sensitivity of colistin-resistant *E. coli* isolates to antibiotics

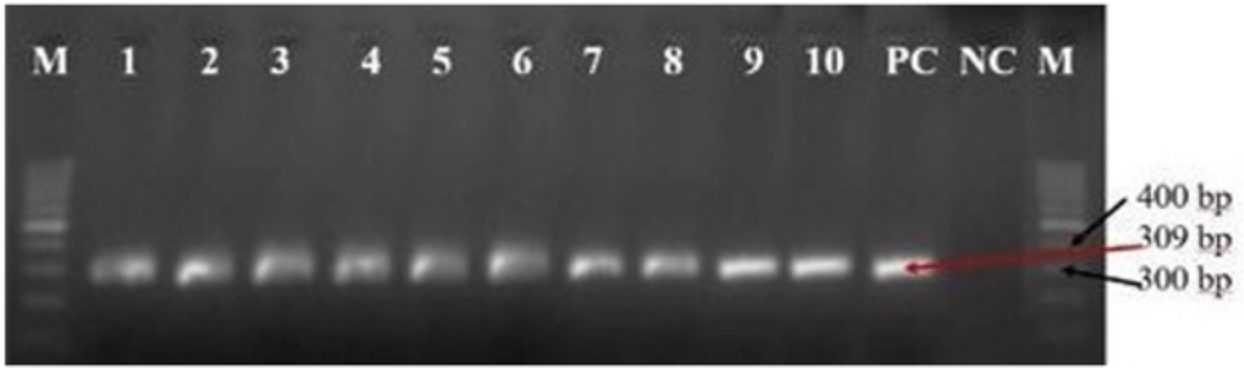

Figure 2. Gel electrophoresis for colistin resistance encoded by the *mcr*-1 gene. M: 100 bp DNA Ladder, 1-10: field isolates with *mcr*-1 gene (309 bp) 11: Positive Control (*E. coli* NCTC 13846), NC: Negative Control (*E. coli* 25922)

bacteria, particularly *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Klebsiella pneumoniae* (Poirel et al., 2017). The discovery of mobile colistin resistance determinants in humans and animals has raised concerns about the future of antimicrobials (Apostolakos and Piccirillo, 2018). Therefore, this study aimed to determine the presence of *mcr*-1, which provides plasmid-mediated colistin resistance in APEC isolates, and to examine the antibiotic resistance profiles of colistin-resistant isolates.

The dissemination of the *mcr*-1 gene to many countries and its presence in bacteria isolated from various environmental sources have been reported (Skov and Monnet, 2016). This situation could increase the transmission of colistin resistance from animal husbandry and agricultural areas to the environment and humans. Therefore, the spread of the *mcr*-1 gene could lead to a serious public health issue.

Studies conducted in food-producing animals have demonstrated an increase in colistin resistance, especially among poultry in Asia (Kempf et al., 2016; Webb et al., 2017). The cheap source of protein provided by poultry and the increasing trend of colistin resistance necessitate a thorough evaluation of colistin use in chickens (Apostolakos and Piccirillo, 2018).

In Türkiye, colistin sulphate is applied to treat gastrointestinal and respiratory system infections caused by *E. coli* and *Salmonella* species in poultry. This study confirms the use of colistin in broiler chickens for therapeutic purposes on the farms where the samples were collected. While studies on colistin resistance in Türkiye are limited (Kurekci et al., 2018; Adıgüzel et al., 2021; Erzaim and İkiz, 2021; Aslantaş and Küçükaltay, 2023; Seferoglu et al, 2024) and there is increasing awareness of this issue. The first reported case of *mcr*-1 positive *E. coli* isolate in the country emerged in Hatay (Kurekci et al., 2018). In a study conducted in Istanbul, phenotypic colistin resistance was detected in 7.5% of 200 *E. coli* isolates obtained from broiler intestinal samples. However, none of the examined samples, including isolates with phenotypic colistin resistance, were found to carry the *mcr*-1 gene. Although colistin resistance was identified phenotypically, the absence of the plasmid-mediated *mcr*-1 gene suggested a chromosomal origin of resistance or the involvement of other resistance genes (Erzaim and İkiz, 2021). Furthermore, a study of colistin resistance in commensal *E. coli* strains isolated from chicken flocks in Hatay revealed that out of 454 isolates examined from cloacal swabs, five isolates carried the *mcr*-1 gene as determined by PCR. Phylogenetic analysis based on whole-genome and multi-locus sequence typing showed that these strains were closely related to *mcr*-1 carrying isolates previously reported from chicken and human clinical isolates in different regions of the world (Aslantaş and Küçükaltay, 2023). In this study, all isolates identified as phenotypically resistant to colistin were found to carry the *mcr*-1 gene, indicating that resistance was plasmid-mediated and mobile. Of the via plasmid transfer of the *mcr*-1 gene can confer colistin resistance to other bacteria, increasing the risk of disease spread. Additionally, the *mcr*-1 gene can be transferred from animal sources to humans, increasing the risk of zoonotic infections. Considering other studies conducted in our country (Adıgüzel et al., 2021; Erzaim and İkiz, 2021), it is evident that colistin resistance persists in isolates obtained from chicken meat in Türkiye, highlighting it as a significant problem over a wide geographical area.

Table 4. The number of antibiotics to which colistin-resistant *E. coli* isolates are resistant, moderately sensitive and sensitive according to the antibiotic susceptibility test

	Number of antibiotics									
Antibiotic sensitivity test result	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Resistance	12	13	9	9	8	11	11	12	13	7
Intermediate	0	0	1	1	1	0	1	1	0	0
Susceptible	7	6	9	9	10	8	7	6	6	12

In veterinary medicine, colistin is commonly used for disease prevention, treatment, or growth promotion (Rhouma et al., 2016). However, the discovery of a plasmid-mediated gene transferable between bacterial species in 2016 raised global concerns. The *mcr*-1 gene, one of the mobile colistin resistance genes, was first discovered in 2016 (Liu et al., 2016), and its resistance development mechanism has not been fully elucidated in many cases (Fukuda et al., 2018). Epidemiological data indicate evidence for the emergence of transferable colistin resistance due to the widespread of colistin use in livestock, with evidence of transfer from animals to humans (Poirel and Nordmann, 2016).

The *mcr*-1 gene is located on a plasmid, a small fragment of DNA that can be transferred from one bacterium to another. The rapid spread potential of the gene to other bacteria increases the likelihood that bacteria resistant to multiple antibiotics will also becoming resistant to colistin as well, underscoring its critical importance in the fight against antibiotic resistance (Tenover, 2006).

Determination of colistin resistance in countries and establishment of gene pools are crucial to identify the prevalence of colistin resistance genes (Etebu and Ukpong, 2016). Phenotypic methods, such as broth and agar microdilution, and disc diffusion methods are commonly used to determine colistin resistance in *E. coli*. However, these methods are time-consuming, impractical, and require laboratory skills. The disc diffusion method provides faster results but lacks standardised disc diffusion zone diameters, making interpretation difficult due to the large molecular structure of colistin (EUCAST, 2022).

Recent studies have reported that automated systems for determining colistin resistance in *E. coli* isolates do not lead to significant errors and demonstrate have acceptable performance (Yiş, 2022; Zhang et al., 2023). The BD Phoenix 100™ automated microbiology system is one of the automated systems used for antimicrobial susceptibility testing. This system uses the broth microdilution method to determine minimum inhibitory concentrations. Due to its automated nature, the BD Phoenix 100™ system can speed up testing processes and reduce operator intervention. Automated systems provide standardised test conditions, this leads to more consistent results. The BD Phoenix 100™ can perform susceptibility testing for many different antibiotics simultaneously (BD Phoenix, 2023). In this study, the BD Phoenix 100™ automated microbiology system was used due to its advantages, such as rapid results for determining phenotypic colistin resistance, standardised test conditions, and multiple antibiotic testing.

The widespread use of antibiotics in poultry farming and the emergence of antibiotic-resistant bacteria pose a significant challenges to both animal and human health. In our study, all isolates were resistance to multiple antibiotics, indicating the limited treatment options available. Similar findings have been reported in previous studies of poultry isolates carrying the *mcr*-1 gene, highlighting the global concern of surrounding antibiotic resistance in poultry production (Apostolakos and Piccirillo, 2018; Aslantas and Kucukaltay, 2023).

Conclusion

In this study, the presence of the *mcr*-1 gene in APEC isolates was confirmed, highlighting the widespread prevalence of colistin resistance and its impact on other antibiotics. All phenotypically colistin-resistant isolates were found to carry the *mcr*-1 gene, indicating plasmid-mediated mobilisation of colistin resistance. These findings highlight the urgent need for surveillance and control measures to address antibiotic resistance in animal pathogens, and emphasise the importance of revising veterinary health policies and regulating the use of colistin. Monitoring the use of antibiotics in animal husbandry and promoting sustainable practices are crucial steps in the fight against antibiotic resistance and the protection of animal and human health. In addition, further research on colistin resistance and genetic spread is essential, highlighting the importance of implementing appropriate measures to prevent the spread of resistance genes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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