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Serotyping of *Escherichia coli* species isolated from broilers and determination of Colistin resistance

Serotipado de *Escherichia coli* aisladas de pollos de engorde y determinación de resistencia a la Colistina

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ABSTRACT

Systemic infections by avian pathogenic *Escherichia coli* (APEC) are economically damaging to poultry industries Worldwide. *E. coli* strains of serotypes O1, O2, O18 and O78 are preferentially associated with avian colibacillosis. The *rfb* gene cluster that controls O antigen synthesis generally varies among different *E. coli* serotypes. In this study, the *rfb* gene clusters of *E. coli* serotypes O1, O2, O18 and O78 were characterized and compared, and it was also aimed to search for Colistin resistance on a molecular basis. For the research, 200 swab samples were taken from 200 chickens suspected of colibacillosis in broiler poultry farms located in the vicinity of Aydın, İzmir, and Manisa Provinces in Turkey 2022. Bacterial growth was obtained from 92% of the samples, and microbiological analysis identified 108 (54%) *Escherichia coli* isolates. In addition, *Klebsiella* spp. was identified in 35 (17.5%) samples, *Proteus* spp. in 23 (11.5%), *Pseudomonas* spp. in 18 (9%), and no bacterial growth was observed in 16 (8%) samples. *mcr-1* (309 bp) and *mcr-2* (567 bp) genes responsible for Colistin resistance was investigated in plasmid DNA extracted from 108 *E. coli* isolates obtained in the study, using the PCR method. However, neither *mcr-1* nor *mcr-2* genes were detected in any of the samples. In conclusion, the allele-specific PCR method was found sensitive and applicable for APEC identification and multiple drug resistance emerged in *E. coli* strains isolated according to the antibiogram results.

Key words: Avian pathogenic *Escherichia coli; mcr* gene; antibiotic resistance

RESUMEN

Las infecciones sistémicas por *Escherichia coli* patógena aviar (APEC) son económicamente dañinas para las industrias avícolas en todo el mundo. Las cepas de *E. coli* de los serotipos O1, O2, O18 y O78 se asocian preferentemente con la colibacilosis aviar. El grupo de genes *rfb* que controla la síntesis del antígeno O generalmente varía entre los diferentes serotipos de *E. coli*. En este estudio, los grupos de genes *rfb* de los serotipos O1, O2, O18 y O78 de *E. coli* se caracterizaron y compararon, y también tuvo como objetivo buscar la resistencia a la Colistina sobre una base molecular. Para la investigación, se tomaron muestras de hisopados de 200 pollos con sospecha de colibacilosis en las granjas avícolas de pollos de engorde en las provincias de Aydın, İzmir y Manisa, en Turkia, 2022. Como resultado del análisis microbiológico de las muestras se identificaron 108 (54 %) *Escherichia coli*. Además, *Klebsiella* spp. en 35 (17,5 %) de las muestras, *Proteus* spp., en 23 (11,5 %) y *Pseudomonas* spp., en 18 (9 %) de las muestras identificadas y el crecimiento bacteriano no ocurrió en 16 (8 %) de ellos. La presencia de los genes *mcr-1* (309 pb) y *mcr-2* (567 pb) responsables de la resistencia a la Colistina en los ADN plasmídicos extraídos de 108 aislados de *E. coli* obtenidos en el estudio, se investigó mediante el método PCR. Como resultado de la amplificación por PCR, los genes *mcr-1* y *mcr-2* no pudieron detectarse en ninguna de las muestras. El método de PCR se evaluó como un método sensible y aplicable para la identificación y serotipificación de APEC, y se determinó el perfil de resistencia a múltiples fármacos en las cepas de *E. coli* aisladas de acuerdo con los resultados del antibiograma.

Palabras clave: *Escherichia coli* patógena aviar; gen *mcr*; resistencia a antibióticos

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INTRODUCTION

Escherichia coli is one of the most important pathogens causing enteritis and septicaemia in various species such as poultry (*Gallus gallus domesticus*), pigs (*Sus scrofa*), ruminants (*Bos taurus*), dogs (*Canis lupus familiaris*), cats (*Felis catus*), horses (*Equus caballus*) and rabbits (*Oryctolagus cuniculus*). *E. coli*, which causes local or systemic infections in poultry, was first isolated by German paediatrician Theodor Escherich and named Escherichia to honour him. Avian pathogenic *E. coli* (APEC) is the cause of colisepticaemia, coligranuloma (Hjarre's disease), air sacculitis, coliform cellulitis, bulging head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/synovitis, coliform panophthalmitis and coliform cheocephalitis [1]. It is known to cause inflammation (yolk sac infection) and colibacillosis is one of the most commonly observed diseases among poultry diseases of bacterial origin. In the 1990s, deaths due to septicaemia were among the most common cases in laying hen farms in Europe. In addition, APEC-related infections in layers and broiler chickens were reported in Belgium between 1997 and 2000 were 17.7 and 38.6%, respectively, and resistance to antibiotics was also high [2, 3].

Serotyping of *E. coli* is due to the O antigen in lipopolysaccharide and the H antigen in flagella. O antigen denotes serogroup and H antigen denotes serotype. Capsular antigen (K) is also used for classification. Somatic O antigens are heat-resistant surface antigens in lipopolysaccharide structure. Lipopolysaccharide, also known as endotoxins, are found on the outer membrane of Gram-negative bacilli cell wall. Somatic O antigens Although they are important in the serogrouping of *E. coli*, and they can be revealed by agglutination test. Flagellar H antigen varies according to the different types of flagellin protein found in the structure of the flagella. H antigen, which is heat labile, can be detected by agglutination test. Capsular K antigen is in polymeric acid structure. They are present on the cell surface and prevent the agglutination of the O antigen. They are divided into L, A and B subclasses according to their heat sensitivity. Fimbrial (*Pilus*) antigens were previously called the L subclass of K antigens, but as a result of the studies, currently is well known they are located in the fimbriae of *E. coli*. The pilus, which plays a role in the adhesion of bacteria to intestinal epithelial cells, is divided into mannose-sensitive and mannose-resistant [2].

Generally, O1, O2, O8, O15, O18, O35, O78, O88, O109 and O115 serogroups of O antigen have been detected in colibacillosis infections of poultry. O1, O2 and O78 were the most prevalently detected serogroups. Pathogenic *E. coli* species are divided into intestinal and extraintestinal *E. coli* pathotypes according to the type of infection. Intestinal pathogenic *E. coli* (IPEC) causing diarrhoea are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAEC). Extraintestinal pathogenic *E. coli* species are divided into uropathogenic *E. coli* (UPEC), septicemia-causing *E. coli* (SePEC), newborn meningitis-causing *E. coli* (NMEC) and avian pathogenic *E. coli* (APEC) [4].

The scope of the research was to analyse the *rfb* gene groups of the dominant serovars of avian pathogenic *E. coli*, including O1, O2, O18, and O78 strains, and to determine the serotyping of O-antigens with an allele-specific Polymerase chain reaction (PCR) method. The sensitivity and applicability of the allele-specific PCR method for APEC identification and serotyping were investigated, and Colistin resistance was searched by molecular methods.

MATERIAL AND METHODS

Animal specimen, isolation methods and primers

For the research, cloacal swab samples were taken from 200 chickens with suspected colibacillosis and natural death from broiler poultry houses around Aydın, İzmir and Manisa Provinces in Turkey 2022. The samples were brought to Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Routine Diagnostic Laboratory in cold chain. Swap samples were incubated at 37°C for 24 h after inoculation on MacConkey agar. Lactose positive pink colonies were seeded on EMB agar and incubated at 37°C overnight. Colonies that gave metallic green sheen on EMB medium after incubation were purified, and *E. coli* was identified by Gram staining, indole, methyl red, Voges-Proskauer, citrate catalase, oxidase reactions and motility examinations after purification. Serotyping with various commercial antisera kits (O1, O2, O5, O6, O8, O9, O11, O12, O14, O15, O17, O18, O20, O35, O36, O45, O53, O78, O81, O83, O88, O102, O103, O115, O116 and O132) (Mast Assure™, UK) was applied to the isolates identified as *E. coli*. O1, O2, O18 and O78 serotypes identified after serotyping according to agglutination reactions were confirmed by allele-specific PCR method. Serotyping isolates and other identified *E. coli* strains were PCR processed with *mcr-1* and *mcr-2* specific primers to determine Colistin resistance. The primer sequences used in the study are shown in TABLE I below [5, 6, 7].

TABLE I
The primer sequences used in the study

Target gene	Primer Sequences (5'-3')	Fragment lenght	Reference
ECO-F (*gnd*)	F: 5'-CGATGTTGAGCGCAAGGTTG-3'		[5]
ECO1-R (*rfb*O1)	R:5'-CATTAGGTGTCTCTGGCACG-3'	263 bp	[5]
ECO2-R (*rfb*O2)	sR:5'-GATAAGGAATGCACATCGCC-3'	355 bp	[5]
ECO18-R (*rfb*O18)	R:5'-AGAAGCATTGAGCTGTGGAC-3'	459 bp	[5]
ECO78-R (*rfb*O78)	R: 5'-TAGGTATTCCTGTTGCGGAG-3'	623 bp	[5]
mcr-1	CLR F: 5'-CGGTCAGTCCGTTTGTTC-3' CLR R: 5'-CTTGGTCGGTCTGTAGGG-3'	309 bp	[6]
mcr-2	MCR2 IF: 5' – TGTTGCTTGTGCCGATTGGA-3' MCR2 IR: 5'-AGATGGTATTGTTGGTTGCTG-3'	567 bp	[7]

Genomic DNA isolation

The deoxyribonucleic acid (DNA) was extracted from pure *E. coli* isolates using a bacterial DNA isolation kit (Fermentas, Lithuania), according to the manufacturer's instructions. All isolation steps were completed in 1.5 mL microcentrifuge tubes.

PCR

In allele-specific PCR for serotyping, the conditions were initial denaturation at 95°C for 5 min, followed by 30 cycles of 35 s denaturation, at 95°C, 30 s annealing at 57°C, 40 s extension at 72°C and, 10 min of final extension at 72°C. For the PCR mix, in a total

volume of 25 µL, 2.5 µL of 10X PCR Buffer (25mM $MgCl_2$), 2 µL of dNTP (2.5 mM of each dNTP), 1.5 U of Taq polymerase, 0.5 µL of each primer (10 µM), and 1 µL of DNA sample was used [5].

In the PCR procedure to determine Colistin resistance, the conditions were initial denaturation at 94°C for 15 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 90 s, extension at 72°C for 60 s and, consisted of a 10 min final extension phase at 72°C. For the PCR mix, 8.5 µL of milliQ water, 12.5 µL of 2X PCR mix, 0.5 µL of each primer (2 µM) and 2 µL of DNA sample were used in a total volume of 25 µL. PCR products were analysed by agarose gel electrophoresis (Agarose-ME, Classic Type; Nacalai Tesque, Inc., Japan), and visualized in a UV device (Infinity VX2, France) . Target electrophoretic bands with specific fragment length were visualized (Mastercycler Personal; Eppendorf, Netheler, Hinz GmbH, Hamburg, Germany). *E. coli* IMT 2467 (serotype O1), *E. coli* IMT 5155 (serotype O2), *E. coli* IMT 663 (serotype O78) were used as positive controls. All these positive controls for the specific genes were obtained from of AniCon Labor GmbH (AniCon Labor GmbH, Emstek, Germany). The positive control strains of Colistin resistance were obtained from NCTC as *E. coli* 14377 (*mcr-1*) and *E. coli* 14378 (*mcr-2*). For the positive control of serotype O18, field strain of department laboratory isolated from chicken were used.

Antibiotic susceptibility test

Antibiotic susceptibility test of the isolates was performed following disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) [8]. A loopful of pure cultures of the identified agents was taken and inoculated into 5 mL of Brain-Heart Infusion broth, and the media were incubated for 24 h at 37°C. The bacterial suspension obtained was adjusted to 0.5 McFarland turbidity and 100 µL was taken and after inoculation on Mueller-Hinton agar, antibiotic discs were placed on the agar at appropriate intervals. After 24 h of incubation (Nüve EN500, Turkey) of the media at 37°C, the zone diameters around the antibiotic discs were measured in millimetric scale and the antibiotic susceptibility of bacterial agents was determined. The antimicrobial agents used in this research were as follows: amikacin (AK), Ampicillin (AMP), Colistin (CL), Cotrimoxazole (SXT), Ciprofloxacin (CIP), Doxycycline (DO), gentamicin (GEN), levofloxacin (LE) and nitrofurantoin (NIT). For quality control of antibiotic susceptibility test, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains [9].

RESULTS AND DISCUSSION

Isolation and identification findings of the serotypes

In this study, *E. coli* was identified from 108 (54%) of 200 cloacal swab samples. In addition, *Klebsiella* spp. from 35 (17.5%) of the samples, *Proteus* spp. from 23 (11.5%) and *Pseudomonas* spp. from 18 (9%) of the samples. Gram negative bacterial growth did not occur in 16 (8%) of them. Bacteria isolated and identified from cloacal swabs from broiler chickens are shown in TABLE II. The distribution of the identified *E. coli* isolates according to O1, O2, O18, and O78 serotypes is shown in TABLE III. The distribution of isolated serotypes by cities is shown in TABLE IV.

PCR findings

PCR gel electrophoresis image of serotypes is given in FIG. 1. In order to determine the genotypic resistance, 108 isolates were extracted by the previously mentioned methods. All of 9 APEC O1, 11 APEC O2, 6 APEC O18 and 39 APEC O78 serotypes isolated in this

TABLE II
Bacteria isolated and identified from cloacal swabs from broiler chickens

Bacterial species	Isolate number	(%)
E. coli spp.	108	54
Klebsiella spp.	35	17.5
Proteus spp.	23	11.5
Pseudomonas spp.	18	9
No bacterial growth	16	8
TOTAL	200	100.00

TABLE III
The distribution of the identified *E. coli* isolates according to O1, O2, O18, and O78 serotypes

Serotypes	APEC isolates (n=108)	
	PCR	Serum agglutination
O1	9	8
O2	11	10
O18	6	6
O78	39	36
O1, O2, O18, O78	0	5
Other serotypes	–	43

TABLE IV
The distribution of isolated serotypes by cities

Cities	*Sample*	*E. coli* O1	*E. coli* O2	*E. coli* O18	*E. coli* O78	*Other*
Manisa	70	5	6	3	17	16
İzmir	70	3	3	3	12	14
Aydın	60	1	2	–	10	13
TOTAL	200	9	11	6	39	43

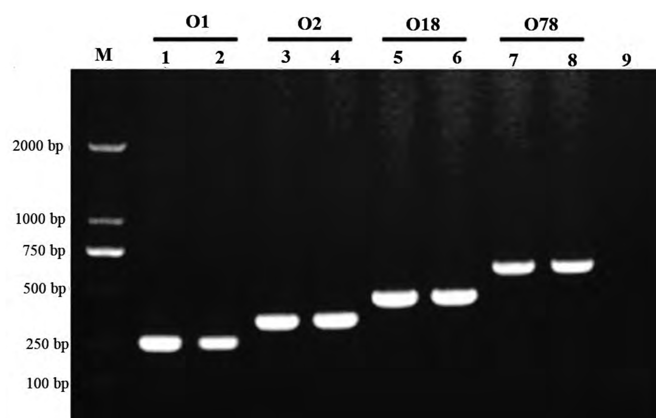

FIGURE 1. PCR gel electrophoresis image of serotypes Gel size of *E. coli* serovars O1, O2, O18 and O78. M: DL2000 DNA Marker; O1, O2, O18 and O78 represent PCR products for serovars O1, O2, O18 and O78, respectively. 1: Positive control APEC IMT2467; 2: Isolate APEC O1; 3: Positive control APEC IMT555; 4: Isolate APEC O2; 5: Positive control APEC Field strain; 6: Isolate APEC O18; 7: Positive control APEC IMT663; 8: Isolate APEC O78; 9: Negative control (*P. aeruginosa* ATCC 27853)

study were confirmed by allele specific PCR test. The results indicated that 65 (60%) of 108 *E. coli* isolates showed corresponding PCR bands at respective size, which was in accordance with bacteriological examination and conventional serum agglutination assay. The presence of *mcr-1* (309 bp) and *mcr-2* (567 bp) genes responsible for Colistin resistance in plasmid DNAs extracted from 108 *E. coli* isolates obtained in the study were investigated by PCR method. As a result of PCR amplification, *mcr-1* and *mcr-2* genes could not be detected in any of the samples. Resistance gene PCR image is given in FIG. 2.

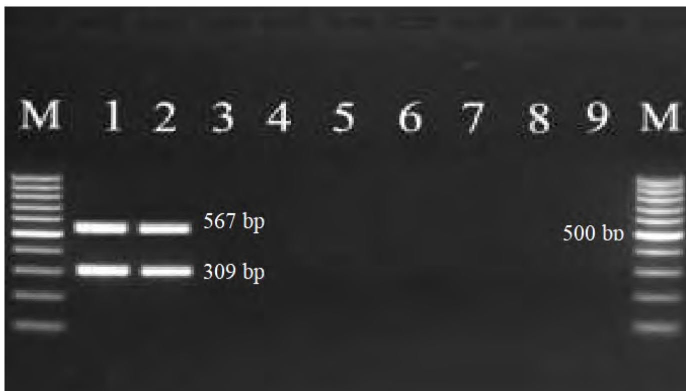

FIGURE 2. Resistance gene PCR image M: Marker 1 and 2: Positive controls (*E. coli* 14377 and 14378) 3: Negative control (*P. aeruginosa* ATCC 27853) 4-9: Isolates studied in this research

Antibiotic susceptibility findings

None of the nine antibiotics tested introduced 100% efficacy versus *E. coli* strains. According to the antibiogram results, 98% of strains were resistant to Ampicillin at the highest rate and 16% of strains were resistant to Amikacin at the lowest rate. Cotrimoxazole, Doxycycline, and Ciprofloxacin were found to be responsible for more than 60% resistance. In addition, 5 (7.6%) of 65 isolates were phenotypically resistant to Colistin.

One of the leading causes of disease and mortality in chickens across the Globe is colibacillosis. The majority of instances of avian colibacillosis are caused by APEC serotypes O1, O2, O18, and O78 strains. In some APEC outbreaks, many strains with varied serotypes have been discovered. Additionally, trans protection among several APEC serovars is weak [10]. Thus, for APEC control, a quick and precise serotyping approach is essential. Conventional APEC serotyping methods like the serum agglutination test require a spectrum of strong antisera versus bacterial isolates and several O-antigens in order to produce results. Serum agglutination cannot differentiate APEC serotypes in a single test. Furthermore, a strain may react with more than one APEC antiserum. Hence, an allele-specific PCR test was applied for serotyping the dominant APEC serotypes in this research. Between the rfb locus and the gnd gene, distinct O-antigen sequences were amplified using PCR in various sizes. This study showed that the PCR test can be applied to serotyping APEC O1, O2, O18, and O78 strains in bacterial cultures and did not react with reference strains of *E. coli* and other bacterial species with different serotypes. Furthermore, multiple agglutination isolates can be clearly serotyped using this method, indicating that the PCR test is specific and reliable.

The PCR test was successful in serotyping APEC strains in clinical infected tissue samples, suggesting its use for laboratory detection. Additionally, it was able to distinguish serotypes of multi-agglutination samples in routine bacteriological examination. Therefore, this PCR test was found to be more accurate than conventional serum agglutination assays. The APEC clinical diagnosis and epidemiological research were made easier with lighter effort and in a short amount of period. To summarize, the study utilized an allele-specific PCR test that had the ability to accurately differentiate between the dominant APEC serotypes (O1, O2, O18, and O78) with great precision and sensitivity. This approach addresses the limitations of traditional serological tests and offers an efficient and suitable method for identifying the predominant APEC strains. Consequently, using this PCR test offers advantages for clinical diagnosis and epidemiological surveys.

One of the most common mechanisms is the acquisition of resistance genes through horizontal gene transfer. This process involves the transfer of genetic material from one bacterium to another, often facilitated by plasmids or other mobile genetic elements. Another mechanism of antibiotic resistance in APEC is the presence of efflux pumps. These pumps are proteins that are able to remove antibiotics from the bacterial cell before they can exert their effects. This mechanism of resistance is particularly common in Fluoroquinolone – resistant APEC strains. Studies have shown that antibiotic resistance is a growing problem in APEC infections. Resistance to Fluoroquinolones and Tetracyclines is particularly common, with some APEC strains showing resistance to multiple antibiotics. In some cases, resistance to one antibiotic can also confer cross-resistance to other antibiotics. For example, resistance to Tetracyclines can often lead to resistance to other antibiotics in the same class, such as Doxycycline and Minocycline. The prevalence of antibiotic resistance in APEC strains varies depending on geographical location and other factors. Antibiotics are widely used in the commercial poultry sector to treat illnesses and encourage growth [11]. Globally, it is estimated that antimicrobial consumption in animal food production will increase by 67% by 2030. In the Asia-Pacific Region, the use of antimicrobials in chickens is expected to increase by 129% by 2030. Avian colibacillosis is the leading disease reported in chickens in several previous studies. The use of antibacterial as a treatment approach in the chicken industry is successful in reducing the danger of colibacillosis, but the introduction of multi-drug resistant bacteria and the transmission of resistance genes have made this task much more difficult [12].

None of the nine tested antibiotics introduced 100% efficacy versus *E. coli* strains. Ampicillin showed the highest resistance rate among *E. coli* isolates, with up to 98% being resistant to it. On the other hand, the lowest resistance rate was observed for Amikacin, with only 16% of the isolates showing resistance to it. Trimethoprim-Sulfamethoxazole, Doxycycline and Ciprofloxacin were responsible for more than 60% of the resistance. *E. coli* serovar patterns of antibiotic resistance were similar to those documented in earlier research [13]. On the other hand, Amikacin was the most successful against 84% of strains, as demonstrated by Bist *et al.* [14]. The improper implementation of various drugs in chicken feed and treatment is a prevalent application [15]. The indiscriminate use of antibiotics applies a selection pressure that leads to the development of drug-resistant bacterial strains. The patterns of antibiotic resistance discovered in this study point to a concerning incidence of resistant *E. coli* serovars in broiler chickens in the Aegean Region of Turkey.

Multi-antibiotic resistance patterns showed that 94% of the isolates were resistant to three or more antimicrobials. The high prevalence of multidrug resistance (MDR) in *E. coli* has been documented in Bangladesh, China, and Korea [16, 17]. The range of serovars with an MAR index higher than 0.2 was 94%, while the proportion of isolates with an MAR index of 0.2 or less was 6%. An MAR index value greater than 0.2 indicates high-risk contamination sources, where various antibiotics can frequently be used to control diseases [18]. This is a strong indicator of the indiscriminate and improper use of drugs. Antibiotic-resistant microbes eventually take the place of antibiotic-sensitive ones in the environment [19].

Colistin is used as a last resort in bacteria that show multiple resistances. Its use has been restricted in many countries due to its negative effects on human health over time. However, currently, the Colistin antibiotic is extensively applied and marketed in chicken breeding. Yet, it is also referred to as a saviour for diseases brought on by germs that are resistant to antibiotics, which is one of the most significant current challenges [20]. The *mcr-1* and *mcr-2* are genes found in *E. coli* that encode enzymes known as Colistin resistance proteins. These genes encode enzymes known as phosphoethanolamine transferases, which modify a component of the bacterial cell membrane called lipid A. This modification reduces the annealing of Colistin to the cell membrane, thereby reducing its effectiveness as an antibiotic. The *mcr-1* and *mcr-2* genes are part of a larger family of genes called the mobilized Colistin resistance (*mcr*) genes. In addition, there is evidence to suggest that the *mcr-1* gene can be transferred between different bacterial species, including those that are normally not considered to be pathogenic. This raises concerns about the potential for the emergence of new pathogenic bacteria that are resistant to multiple antibiotics. Bacterial resistance to different antibiotics is developing quickly, and worrisomely, Colistin has recently begun to appear on this list of medications. Although there have not been many instances of Colistin resistance in Turkey yet, it is thought to be a potential emergence in the upcoming years. Bacteria develop resistance to antibiotics through unique systems and mechanisms, and then spread them [21]. Hence, it is important to conduct studies targeted at detecting Colistin resistance, building a Worldwide and local gene pool for it, and figuring out how common the essential gene is.

For genomic characterization of Colistin resistance, PCR techniques are frequently used. Spectrofluorometry (Lumina, Thermo Fischer Scientific, USA), MALDI-TOF MS, microarray, and multiplex real-time PCR methods are future detection methods predicted to be used [22]. However, Irrgang *et al.* [23] suggested that the newly developed TaqMan-based real-time PCR could effectively and quickly detect the *mcr-1* gene. Liu *et al.* [6] reported the first occurrence of plasmid-mediated Colistin resistance with the *mcr-1* gene in *E. coli* and *Klebsiella* spp. The *mcr-1* gene was found in 14.9% of commercially available pork and chicken meats, 20.6% of their samples from pigs, and 1.4% of their isolates from patients in China during the identical study. Research reported afterwards in many nations have revealed that the *mcr-1* gene is becoming more widespread globally [7, 24, 25, 26, 27, 28, 29, 30, 31, 32].

In this study, resistance was observed in 5 of the 65 isolates examined (7.6%) phenotypically. The recorded rate can be regarded as noteworthy even though it is in accordance with published data. The fifth-most utilized veterinary antibiotic in the European Union, according to reports, is Colistin [33]. Colistin is prevalently applied in Turkey. This circumstance can be interpreted as the cause of the reported rate in our investigation. The lack of the pertinent genes encoding (*mcr-1* and *2*), meanwhile, raises the possibility that the resistance is chromosomal or that there are additional genes that contribute to the resistance.

Corresponding to this, researchers in Iran examined at the Colistin resistance-causing *mcr-1*, *mcr-2*, and plasmid encoded *crrB* genes. The research examined nine hundred bacterial isolates, and 3.33% of them showed phenotypic Colistin resistance. However, no plasmid-mediated resistance-providing *mcr-1* and *mcr-2* genes were found in any of the samples studied. The authors agreed that the *phoP* and *phoQ* genes on the chromosome is possibly to reason for the Colistin resistance observed in Northwest Iran. APEC strains isolated from turkeys showed high levels of resistance to Fluoroquinolones and Tetracyclines. Antibiotic resistance in APEC can have significant consequences for the health of poultry and the economic viability of the poultry industry. Infections caused by antibiotic-resistant APEC strains are often more difficult to treat, leading to higher morbidity and mortality rates in affected birds [13, 34].

This, in turn, can lead to significant economic losses for poultry producers. In addition to the impact on animal health, antibiotic-resistant APEC strains also pose a potential risk to human health. There is evidence to suggest that APEC strains can be transmitted from poultry to humans, and that these strains may carry resistance genes that can make infections difficult to treat. In some cases, infections caused by antibiotic-resistant APEC strains have been associated with increased morbidity and mortality in humans [34]. Recently, conducted studies have shown that the *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-7.1*, and *mcr-8* genes encoding phosphoethanolamine transferase enzyme are also responsible for plasmid-mediated Colistin resistance [35].

CONCLUSIONS

As a result, the most prevalent serotype among *E. coli* isolates from chickens was serotype O78. The results also confirmed that there is a variety of APEC strains in the poultry population. The PCR assay used in this study, was able to differentiate APEC predominant serotypes of O1, O2, O18 and O78 strains in accordance with serological methods. Thus, implementation of this PCR assay benefits for clinical diagnostics, epidemiology studies, and disease control.

This APEC strains isolated in this study showed high prevalence of resistance against Ampicillin, Cotrimoxazole, Doxycycline and Ciprofloxacin isolated from the colibacillosis suspected broiler chickens. However, none of the isolates were tested positive for the *mcr-1* and *mcr-2* genes. The absence of the *mcr-1* gene, which is often responsible for plasmid-mediated transfer, is considered promising for the Country. However, considering the present study as a pilot study, it is important to conduct studies with a large number of samples to investigate newly reported genes responsible for Colistin resistance. Colistin is an important antibiotic, especially as a last resort in both medical and veterinary fields. Therefore, it is thought that studies in the field of Veterinary Medicine, aimed at limiting the use of Colistin and determining its place in the Global problem of antibiotic resistance, are crucial. Another approach should be improving surveillance and monitoring of multidrug-resistant bacteria. This would involve the development of rapid diagnostic tests to identify patients with Colistin-resistant infections, as well as the implementation of surveillance programs to track the spread of multidrug-resistant bacteria in healthcare settings

and the environment. The development of new antibiotics and alternative therapies are also important strategies for managing Colistin resistant bacteria. Routine monitoring and screening of the antibiotic resistance of avian pathogenic *E. coli* strains are crucial for operating intervention programs to reduce risk of colibacillosis. A holistic application is required for the prevention and the control of avian colibacillosis in Western Cities and other regions of Turkey.

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Conflict of interest

The authors declare that they have no conflict of interest.

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