Investigation and Molecular Characterization of Colistin Resistance in Commensal *Escherichia coli* Strains Isolated from Broiler Flocks

Aslantaş, Ö.[1,a (*)] and Küçükaltay, K.[2,b]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye
[2] Informatics Institute, Middle East Technical University, Ankara, Türkiye
ORCIDs: [a]0000-0003-0407-8633; [b]0000-0001-6074-8940

* **Correspondence:** Prof. Dr. Özkan ASLANTAŞ, Department of Microbiology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, 31034-Antakya/ Hatay/Türkiye; E-mail: ozkanaslantas@yahoo.com; Telephone: +903262458545/115 23; Fax : +903262455704

ABSTRACT

The emergence and dissemination of plasmid-mediated colistin resistance pose a serious threat to global public health. In this study, a total of 454 commensal *Escherichia coli* strains isolated from cloacal swabs taken from broiler flocks between 2013 and 2014 were first screened for phenotypic colistin resistance by the broth microdilution method. Twelve phenotypically colistin-resistant isolates were screened for *mcr*-1 to *mcr*-5 genes by multiplex PCR. Five of these isolates were found to carry the *mcr*-1 by PCR. Whole-genome sequencing of the *mcr*-1 carrying isolates revealed the presence of additional antimicrobial resistance genes and chromosomal *gyr*A and *par*C mutations conferring resistance to fluoroquinolones. Analysis of virulence gene content revealed 5-20 virulence genes in the isolates and two isolates were defined as avian enteropathogenic *E. coli* (APEC). Phylogenetic analysis based on whole-genome and multilocus sequence typing revealed that the strains were closely related to *mcr*-1 carrying isolates from chicken and human clinical isolates previously reported from different parts of the World. The findings further emphasize the urgent need to implement effective control programs to prevent the emergence and dissemination of colistin-resistant bacteria.

Keywords: Broiler; Colistin resistance; *Escherichia coli*; *mcr-1*; WGS.

INTRODUCTION

The increasing trend of antimicrobial resistance (AMR) among the members of the Enterobacterales has become a global health concern. It has been well documented that the widespread use of antimicrobials in food-producing animals has become a possible source of horizontal transfer of antimicrobial resistance genes (ARGs) to human pathogens or direct transfer of AMR bacteria (1, 2). The recent discovery of the plasmid-mediated transferable colistin resistance gene *mcr*-1 in *Escherichia coli* isolates of animal and human origin in China in late 2015 by Liu *et al*. (3) caused global concern, as colistin is the last resort antimicrobial used for the treatment of serious infections caused by MDR Gram-negative bacteria (4). Before this date, colistin resistance was thought to be associated with mutations in the two-component system (*pmr*AB, *pho*PQ) and the regulatory *mgr*B gene, leading to the modification of the chemical structure of lipid A-moieties (3). The use of colistin for treatment or prophylaxis purposes in animals had been considered as a cause of the emergence of the plasmid-mediated colistin resistance, and this has led to the ban of the use of colistin in food-producing animals worldwide (5-7).

Polymyxins (polymyxin E (colistin) and polymyxin B) belong to the family of cationic polypeptide antibiotics with lipophilic fatty acyl side chains (8, 9). The electrostatic interaction between the positively charged polymyxin and the negatively charged phosphate groups of lipid A of lipopolysaccharide (LPS) contributes to the initial binding of

polymyxins to the bacterial surface. Following its diffusion from the outer membrane across the periplasm, polymyxin intercalates into the cytoplasmic membrane to form pores, resulting in the bacteria's lysis (9). Although they belong to the older generation of antibiotics, polymyxins represent the last treatment option for lethal infections caused by Gram-negative pathogens with pan-drug resistant (10).

Following the first report of *mcr*-1 mediated colistin resistance in animal and human *E. coli* isolates (3), various variants of *mcr*-1 (*mcr*-2 to *mcr*-10) have been detected in various bacterial species from different sources reported in the World (11-19). Hu *et al*. (20) suggested that the global spread of the *mcr*-1 gene and its variants may be associated with a food chain-based distribution pathway. Researchers have simultaneously observed the presence of *mcr*-1 in meat and food samples and in the healthy human microbiome. Of concern, the *mcr*-1 colistin resistance gene has been linked to other multidrug resistance genes namely carbapenem (21, 22) and broad-spectrum beta-lactam resistance (23-27) and it has been suggested that this may result in the emergence of pan-drug resistant microorganisms in the future (28). The presence of *mcr-1* gene in Türkiye was first reported in 2018 by Kurekci *et al*. (29), who detected the gene in three *E. coli* isolates obtained from raw chicken meat sold in the retail market. In another study, Adıgüzel *et al*. (30) reported the presence of this gene in an *E. coli* isolate from raw chicken meat in the retail market.

This study aimed to screen *E. coli* isolates from different broiler flocks in southern Türkiye between 2013 and 2014 for the existence of phenotypic and genotypic (*mcr*-1 to *mcr*-5) colistin resistance. To better evaluate the isolates, whole-genome sequencing and phylogenetic analysis were also performed.

MATERIALS AND METHODS

Ethical Statement

Since the isolates used in the study were from author's previous studies, the study does not require ethical approval.

E. coli isolates

A total of 454 non-dublicate commensal *E. coli* strains, which were isolated from cloacal swabs from 20 different broiler flocks in southern Türkiye between 2013-2014, were included in the study. The isolates were obtained from cloacal swabs.

Antimicrobial susceptibility testing

Minimal inhibitory concentration (MIC) for colistin (Sigma-Aldrich, St. Louis, MO) was screened by broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (31). Twofold serial dilutions (128-0.125 µl/ml) of colistin were prepared with cation-adjusted Mueller-Hinton Broth II (Merck, Darmstadt, Germany). Overnight grown fresh bacterial cultures in tryptic soy broth (TSB, Darmstadt, Germany) were diluted at a density of McFarland 0.5 standard. These bacterial suspensions were further diluted 1:100 with TSB and transferred to the wells in 50 µl volumes. To monitor the quality control of the experiment, the strain of *E. coli* NCTC 13846 (*mcr*-1 positive) and susceptible strain of *E. coli* ATCC 25922 were used. Following incubation at 37°C for 24 hr, the lowest antimicrobial concentration with no visible growth was defined as MIC value. A MIC value of >2 µg/ml was accepted as resistance to colistin.

The antimicrobial susceptibility profiles of the *mcr*-1 carrying *E. coli* isolates to 21 additional antimicrobials were determined using the disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) criteria (32). Ampicillin (AM), amoxicillin-clavulanic acid (AMC), cefepime (FEB), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), cefuroxime (CXM), cefotetan (CTT), ceftriaxone (CRO), aztreonam (ATM), ciprofloxacin (CIP), nalidixic acid (NA), imipenem (IPM), chloramphenicol (C), gentamicin (CN), tobramycin (TOB), amikacin (AK), streptomycin (S), kanamycin (K), tetracycline (TE), and sulfamethoxazole-trimethoprim (SXT) were tested for susceptibility testing. The *E. coli* ATCC 25922 was used as a quality control strain.

PCR screening for *mcr* genes

The presence of *mcr*-1 to -5 genes in phenotypically positive *E. coli* (n=5) isolates was screened by a multiplex PCR as previously described (33). *E. coli* (2012-60 for *mcr*-1; KP37 for *mcr*-2; SQ352 for *mcr*-3; DH5a for *mcr*-4) and *Salmonella Paratyphi* B (13-SA01718 for *mcr*-5) were used as positive controls in mPCR reactions.

Whole-genome sequencing (WGS) and analysis

The genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The

quantity and quality of the extracted DNA were measured by spectrophotometric (NanodropOne, Thermo Fisher Scientific) and fluorometric (Qubit 3.0, Thermo Fisher Scientific) methods. Library preparation for short-read sequencing was performed with the use of the NexteraXT kit (Illumina) and dual-indexing system (Illumina) in accordance with the manufacturer's recommendation. Quality and quantity of libraries were confirmed by capillary gel electrophoresis (DNF-473 Standard Sensitivity NGS Fragment Analysis Kit, Fragment Analyzer, Agilent) and fluorimeter (BR assay kit, Qubit 3.0, Thermo Fisher Scientific). The paired-end (2x150 bp) sequencing was performed on the NovaSeq platform (Illumina, San Diego, USA).

Quality checking and *de novo* assembly of sequencing reads

After trimming of low-quality reads and removing adapter sequences using Trimmomatic v 0.36 (34), both raw reads and trimmed reads were checked for quality using FastQC v. 0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/; accessed on 15 January 2021). The *de novo* genome assembly was conducted using the SPAdes algorithm (v 3.14.1) with default parameters (35). Quality assessments of genome assemblies were evaluated using QUAST 4.5 (36). Assembled genomes were analyzed to identify acquired antimicrobial resistance and virulence genes, plasmid types using tools at the Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/). Serotypes and mutation-based resistance genes were searched by CGE web tools. Gene predictions and annotations of the *de novo* assembled genomes were annotated using NCBI Prokaryotic Genome Annotation Pipeline for annotation (37). Core and accessory genome comparison of the isolates was performed using Roary: the pan-genome pipeline (38). Phylogroups were determined by the ClermonTyper at http://clermontyping.iame-research.center (39). The genome assembly data were deposited at NCBI under accession numbers: JAHVAM000000000, JAHVVG000000000, JAHVVH000000000, JAHVVI000000000, and JAHVVJ000000000.

Phylogenetic analysis

For the phylogenetic comparison of our isolates, WGS of 45 *mcr-1* positive *E. coli* isolates of animal and human origin from different countries were retrieved from PATRIC and NCBI databases. *E. coli* K12-MG1655 was also included in the phylogenetic analysis. The tree constructed using the presence and absence of accessory genes were provided in Roary outputs (accessory_binary_genes.fa.newick). The phylogenetic tree was visualized using an interactive web tool iTOL (40).

RESULTS

Prevalence of *mcr* gene in *E. coli* isolates

Overall, five *E. coli* isolates displayed (1.1%) MIC values for colistin ranging from 8 μg/ml to 32 μg/ml. These isolates were found to carry the plasmid-mediated *mcr*-1 gene. The CGE ResFinder tool confirmed the presence of the *mcr*-1.1 gene in all PCR-confirmed isolates. No *mcr*-2, *mcr*-3, *mcr*-4, or *mcr*-5 was detected by either PCR or genome analysis of *mcr*-1 positive isolates.

Genome assembly and annotation

The sequence reads of five *E. coli* isolates were assembled into draft genomes using SPAdes. The median length of the assembly was 5.37 Mbp with an average GC% of 50.4±0.138. The average N_{50} of the assembled contigs was 1.43 Mbp. Annotation of the 5 draft *E. coli* genomes predicted a median number of 5256,8 coding sequences (CDs) (ranging from 5014 to 5534). The median number of contigs per assembly was 152.8 with a minimum of 137 and a maximum of 169 (Table 1).

Core and pangenome comparison

The degree of genomic flexibility of the 5 *mcr*-1-positive *E. coli* strains was evaluated by comparing the core and pangenome structure of the isolates. The overall pangenome consisted of 5662 genes. Of the genes identified by Roary, 61.3% (n=3471) and 38.7% (n=2191) were found in ≥99% of the isolates (referred as core genes) and between 15 and 95% of the isolates (referred as shell genes), respectively (Figure. 1a). The genome comparison of *mcr*-1 positive *E. coli* isolates based on the presence or absence of a gene is shown in Figure 1b. Phylogenetic comparison based on the concatenate core gene alignment also showed genomic diversity among the *mcr-1* positive *E. coli* isolates (Figure 1c).

Table 1. Annotation summary of 5 *mcr-1* carrying *E. coli* isolates by Prokka

Isolate ID	Contigs	Largest contig	Total length	GC (%)	N50	N75	L50	L75	CDS	tRNA	rRNA
HMKU_VET3	137	243 386	5 143 592	50.41	93 494	48 204	19	38	5014	74	–
HMKU_VET4	169	338 726	5 420 389	50.46	177 972	86 712	12	22	5264	69	1
HMKU_VET5	169	338 726	5 420 389	50.46	177 972	86 712	12	22	5264	69	1
HMKU_VET6	145	373 169	5 374 676	50.15	147 917	71 351	10	22	5534	75	3
HMKU_VET7	144	288 669	5 240 238	50.48	116 561	53 118	15	33	5208	71	1
Average	152,8	316 535,2	5 319 856,8	50,4	142 783,2	69 219,4	13,6	27,4	5 256,8	71,6	1,2
Median	145	338 726	5 374 676	50,5	147 917	71 351	12	22	5 264	71	1
Standard deviation	15,1	50 792,7	123 112,8	0,138	37 482,96	18 148,2	3,5	7,6	185,9	2,8	1,1
Minimum	137	243386	5 143 592	50,2	93 494	48 204	10	22	5 014	69	1
Maximum	169	373169	5 420 389	50,5	177 972	86 712	19	38	5 534	75	3

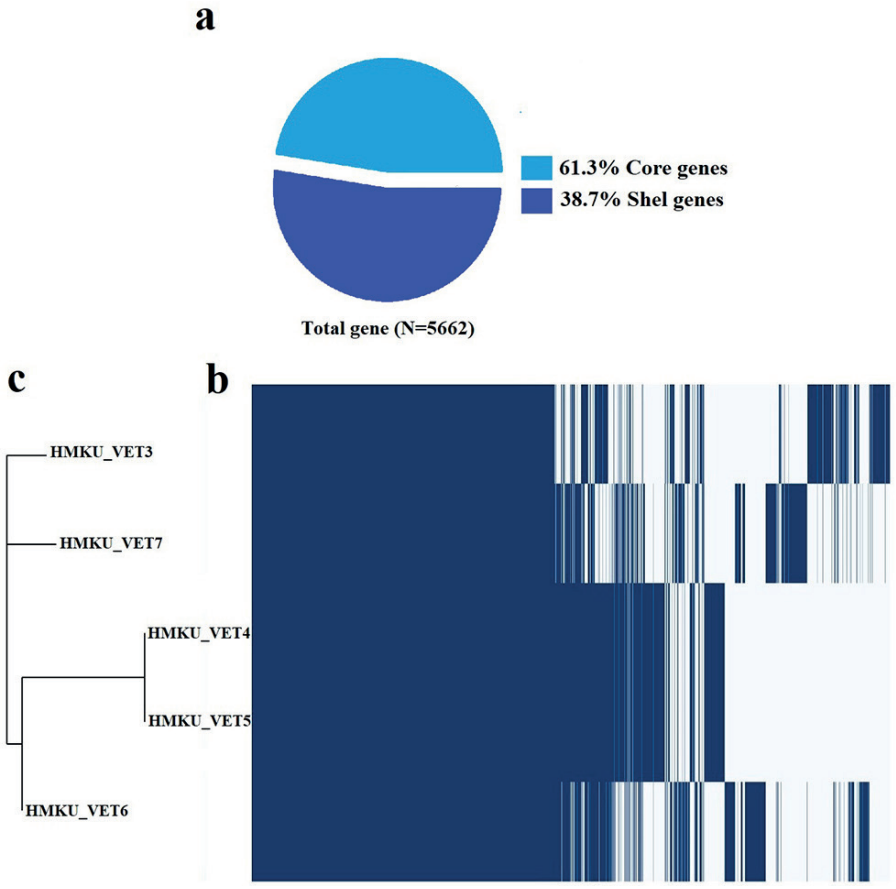

Figure 1. Pangenome comparison of *mcr-1* positive *E. coli* isolates. (a) Distribution of total genes: core genes when found in ≥99%, shell genes when found between 15 and 95% of the isolates. (b) Gene presence (blue) absence (white) matrix across the genome of the isolates. Each row represents the gene content of the respective isolate. Each column represents the possession of homologous gene clusters. (c) Maximum likelihood phylogenetic tree inferred from 3470 concatenated core gene alignment of 5 *E. coli* genome. The tree was constructed by RAxML with 1000 bootstrap using the GRT+gamma model. The data was visualized using Phandango.

Table 2. Genomic features of commensal *E. coli* isolates and their antimicrobial resistance phenotype

Isolate ID	MLST type	Plasmids	Virulence genes*	Resistance Phenotype	Serotypes	Phylogroups
HMKU-VET3	ST1140	IncHI2, IncHI2A, IncY	*chu*A, *gad, hra, omp*T, *ter*C	AM, S, K, CN, TOB, C, NA, SXT, CIP	O38:H39	E
HMKU-VET4	ST88	ColpVC, IncFIB, IncFIC(FII), IncHI2, IncHI2A, IncN	*ast*A, *cea*, *cva*C, *etsC*, *fyu*A, *gad*, *hly*F, *iha*, *iro*N, *irp*2, *iss*, *iuc*C, iutA, *lgf*A, *mch*F, *omp*T, *sit*A, *ter*C, *tra*T, *tsh*	AM, SXT, TE, S, K, NA, CIP	O8:H4	C
HMKU-VET5	ST88	ColpVC, IncFIB, IncFIC(FII), IncHI2, IncHI2A, IncN	*ast*A, *cea*, *cva*C, *ets*C, *fyu*A, *gad*, *hly*F, *iha*, *iro*N, *irp*2, *iss*, *iuc*C, *iut*A, *lgf*A, *mch*F, *omp*T, *sit*A, *ter*C, *tra*T, *tsh*	AM, SXT, TE, S, K, NA, CIP	O8:H4	C
HMKU-VET6	ST10	IncFIB, IncFIC, IncHI2, IncHI2A, IncI-l(Alpha), IncQ1, IncX1	*cib, hra, iss, ter*C, *tra*T	AMP, CPD, AMC, CAZ, CTX, CRO, CTT, CXM, FOX, C, SXT, TE, S, K, CN, TOB, NA, CIP	O88:H12	A
HMKU-VET7	ST156	IncFIB, IncFI, IncI-l(Alpha), IncQ1	cia, *cva*C, *ets*C, *gad*, *hly*F, *hra*, iha, iroN, iss, *iuc*C, lpfA, *mch*B, *mch*C, *mch*F, *omp*T, *pap*C, sitA, *ter*C, *tra*T	AMP, CPD, AMC, CAZ, CTX, CRO, CTT, CXM, FOX, SXT, TE, S, K, NA, CIP	O(-):H51	B1

* *ast*A: EAST-1 heat-stable toxin, *cea*: Colicin E1, *cva*C: Microcin C, *ets*C: Putative type I secretion outer membrane protein, *fyu*A: Siderophore receptor, *gad*: Glutamate decarboxylase, *hly*F: Hemolysin F, *iha*: Adherence protein, *iro*N: Enterobactin siderophore receptor protein; *irp*2: High molecular weight protein 2 non-ribosomal peptide synthetase, *iss*: Increased serum survival, *iuc*C: Aerobactin synthetase, *iut*A:Ferric aerobactin receptor, *lpf*A: Long polar fimbriae, *mch*F: ABC transporter protein MchF, *omp*T: Outer membrane protease (protein protease 7), *sit*A: Iron transport protein, *ter*C: Tellurium ion resistance protein, *tra*T: Outer membrane protein complement resistance, *tsh*: Temperature-sensitive hemagglutinin.

Virulence genes harbored by *mcr*-1 carrying *E. coli* isolates

All isolates harbored multiple virulence genes ranging from 5 to 20. Two isolates carried 20 virulence genes, 2 isolates carried 5 virulence genes and one isolate carried 19 virulence genes. Two isolates carried combinations of virulence genes known to be characteristic for avian pathogenic *E. coli* (APEC) pathotype with an ST88 (Table 2).

In slico MLSTs, serotypes, phylogroup and plasmid genotyping

The *mcr*-1 carrying isolates were found to belong to four phylogenetic groups: E, C (two isolates), A, and B1. We identified four different sequence types (STs) among the 5 *E. coli* isolates. The ST88 was determined in two isolates, other ST types were ST1140, ST10, ST156. *In silico* serotyping also revealed three complete serotypes (O8:H4, O88:H12, O38:H39) and one isolate, based on the H type, was O(-):H51. We identified 12 plasmid replicons in the 5 isolates. All isolates harbored multiple plasmid replicons ranging from 3 to 7. The most abundant replicon types were IncHI2, IncHI2A, and IncFIB which were found in three isolates together, and some of these replicon types were also found individually in other isolates (Table 2).

High prevalence of antimicrobial resistance determinants

We identified a total of 35 different antimicrobial resistance determinants in the 5 strains (Fig. 2). The most abundant antimicrobial resistance genes were *mdf(*A), *qac*E, and *bla*$_{\text{TEM-1}}$, which were found in all isolates. All 5 isolates carried multiple acquired antimicrobial resistance genes with a minimum of 13 to a maximum of 16 genes (Fig. 2a). The isolates exhibited at least one of four known point mutations in two-resistance determinants (*gyr*A, *par*C) that mediate nalidixic acid and quinolone resistance. No mutation was detected in the *par*E gene (Fig. 2b). No known colistin resistance associated with point mutations in *pmr*A and *pmr*B genes was detected among the 5 five *mcr*-1 carrying isolates.

Phylogenetic position of *mcr*-1 positive *E. coli* at the global population structure

The WGS of *mcr-1* positive strains identified in the current study were compared to a global collection originated from Argentina (one human isolate), Bolivia (one human isolate), Brazil (one human isolate), China (one chicken, two human, three migratory birds), Columbia (one human isolate), Egypt (one chicken isolate), France (one human isolate), Germany (one cat isolate, one chicken isolate, one dog isolate, one

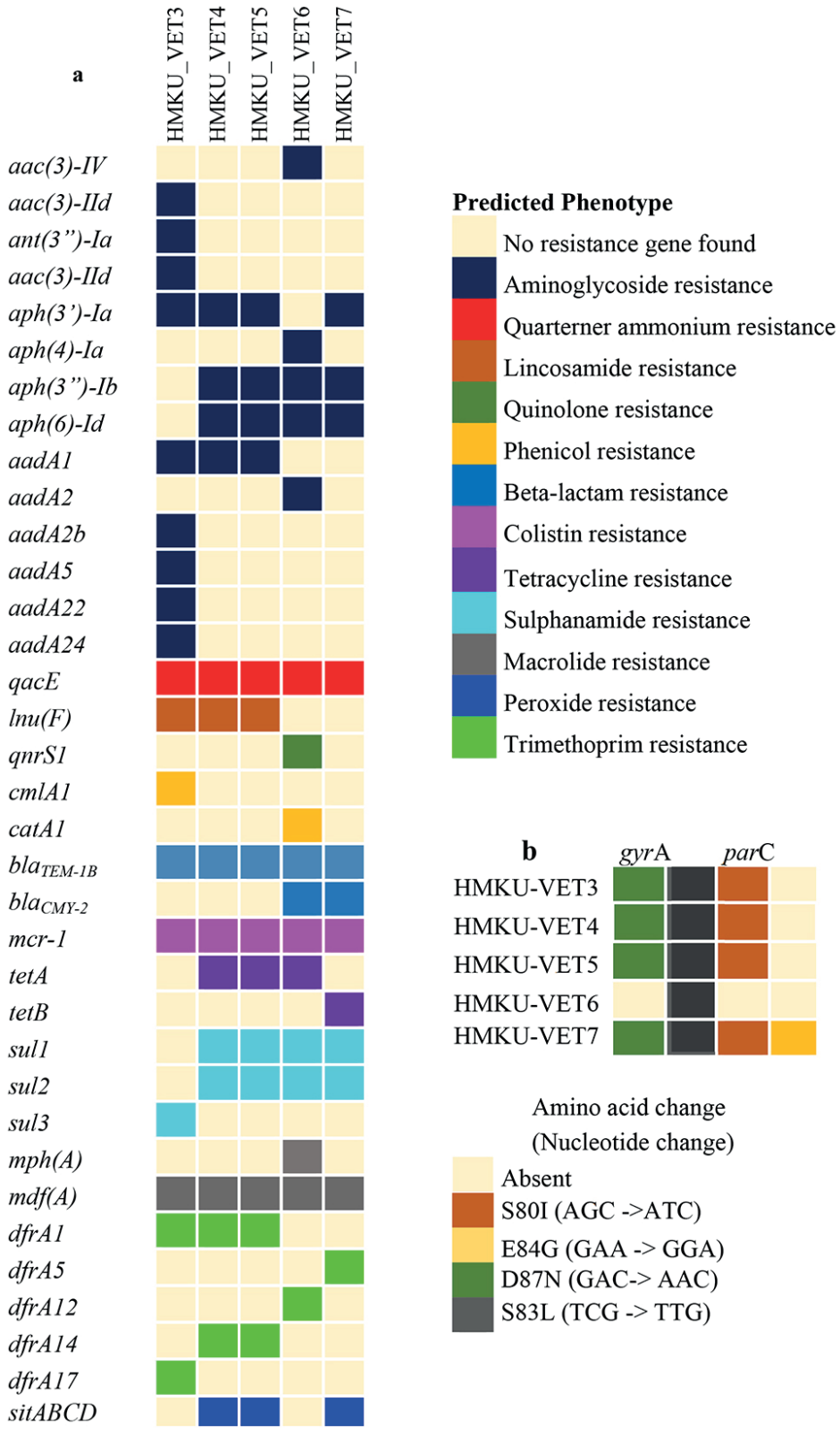

Figure 2. Antimicrobial resistance determinants identified in the genomes of *mcr-1* positive *E. coli* isolates. (a) Heatmap shows the presence or absence of antimicrobial resistance determinants of the isolates. The antimicrobial resistance genes (ARGs) are predicted from whole-genome sequence data using Resfinder 3.2 tool on CGE (b) Heatmap represents chromosomal known point mutation in the *E. coli* genome. Colors indicate amino acid substitution at the corresponding mutation site. Rows and columns represent isolates and mutated genes, respectively.

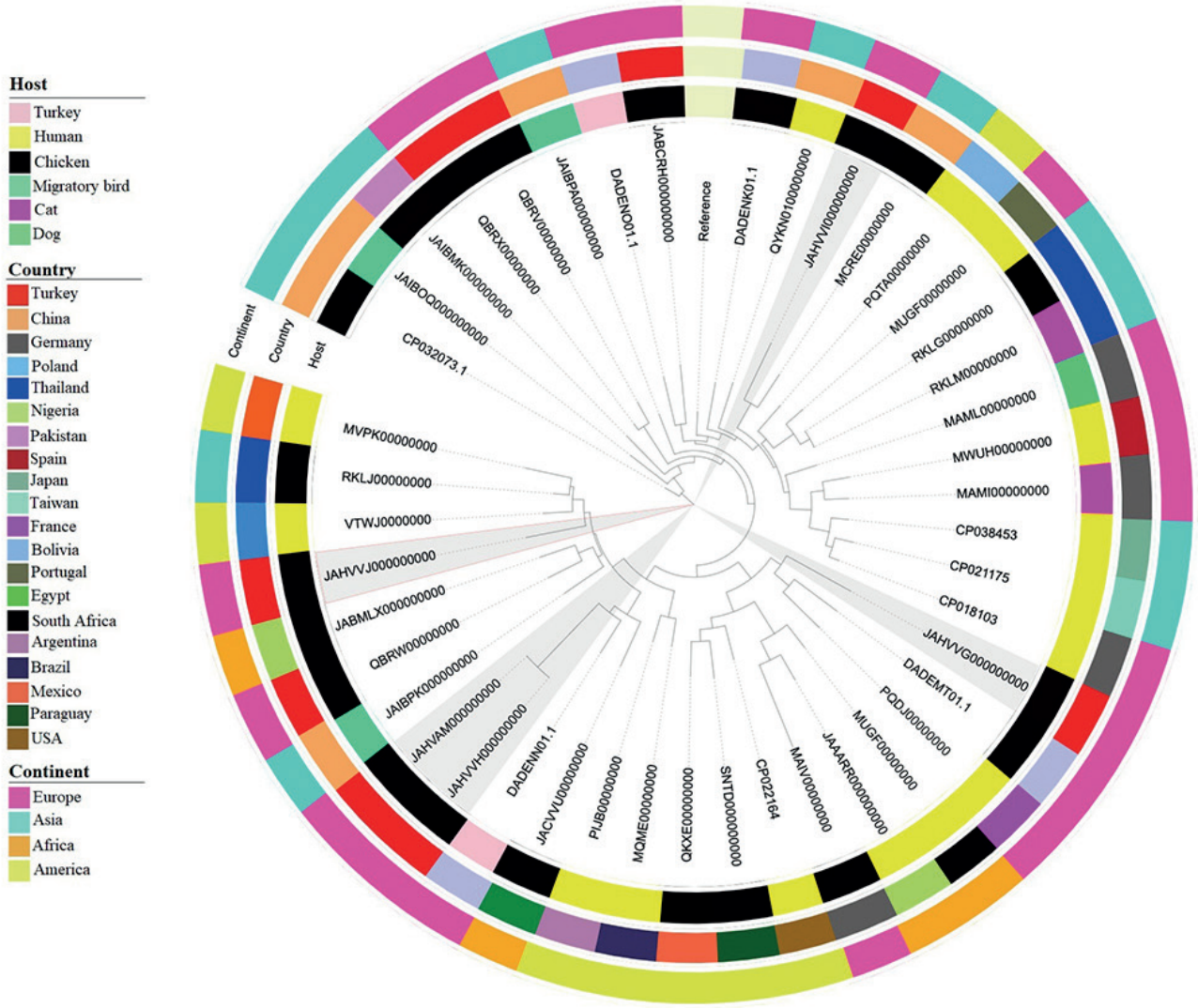

Figure 3. The phylogenetic tree based on the core genome of the *mcr-1* harboring 45 *E. coli* genomes from different regions of the world. Isolates from this study are highlighted with color shade. *E. coli* K12-MG1655 was used as a reference strain.

human isolate), Nigeria (one human isolate, one chicken isolate), Mexico (one human isolate), Japan (one isolate), Pakistan (one chicken), Paraguay (one chicken isolate), Peru (one human isolate), Poland (two chicken isolates, two Türkiye isolates), Portugal (one human isolate), South Africa (one human isolate), Spain (one human isolate), Taiwan (one human isolate), Thailand (two human isolates, one cat isolate, one human isolate) and the USA (one human isolate) (48). The core genome phylogenetic tree displayed three main clades. The tree indicated that *mcr-1* carrying isolates from this study was genetically distinct from the other *mcr-1* carrying *E. coli* strains previously isolated from Türkiye (Fig. 3).

DISCUSSION

Based on the phenotypic and genotypic screening of colistin resistance in *E. coli* isolates, five (1.1%) isolates were found to carry the *mcr*-1 gene and showed 100% nucleotide identity with the previously annotated *mcr-1.1* gene (3). However, *mcr-1* carrying contings (average: 2547.6 bp) were short assemblies that could not be scaffolded to large regions of either plasmids or the chromosome, therefore, their plasmid types were not determined and did not map with any known plasmid available from the GenBank database. Although *mcr* genes have been detected in plasmids belonging to diverse incompatibility (Inc) types, IncX4, InHI2, and IncI2 have been reported as the most common types identified (41-43).

In previous studies conducted in Türkiye, Adıgüzel *et al*. (30) and Kürekci *et al.* (29) reported that *mcr-1* were associated with IncI2 and IncX4 plasmids, respectively. Complete genetic characterization of *mcr*-1-bearing plasmids in *E. coli* isolates is needed using further studies such as bacterial conjugation, transformation, complete whole-genome sequencing.

Worldwide dissemination of the *mcr*-1 gene in several species in Enterobacterales, mostly in *E. coli*, from different animal species and humans, has been attributed to the carriage of this gene on plasmids, which allows for its horizontal transfer (44). Moreover, *mcr-1* bearing plasmids have been reported to have multiple resistance elements in various combinations, alongside *mcr*-1 (41, 45). Since contigs carrying *mcr*-1 were small assemblies in this study, it is not possible to ascertain concerning the presence of other antimicrobial resistance genes or not. However, the results of the current study revealed the presence of multiple antimicrobial resistance genes. Previous studies have also demonstrated multiple antimicrobial resistance genes together with the *mcr*-1 gene (29, 30, 43). Cao *et al*. (46) suggested that the co-existence of *mcr*-1 with other antimicrobial resistance genes may be a contributing factor in the co-selection of *mcr*-1 positive *E. coli* and its spread in broiler flocks/farms. These findings suggest that *mcr*-1 positive *E. coli* should be considered as an important reservoir not only for the colistin resistance gene but also that of other genes conferring resistance to beta-lactams, cephalosporins, aminoglycosides, tetracyclines, sulphonamides, plasmid-mediated quinolone resistance (PMQR). Genomic analysis also revealed that single or multiple mutations present in the *gyr*A gene and the *par*C gene in all isolates, conferring resistance to nalidixic acid and fluoroquinolones. Especially double mutations, which were observed in four isolates, in *gyr*A altering serin 83 to leucine (S83L) and aspartic acid 87 to asparagine (D87N) have been reported to confer a high level of resistance to fluoroquinolones (47).

Pangenome analysis of 5 *mcr*-1 carrying *E. coli* isolates showed that the core genome comprised more than half of the pangenome. In contrast, in a recent study, four times smaller core genome than the pangenome has been reported in *mcr*-bearing *E. coli* isolates (48). Park *et al.* (49) have noted that the size of the core genome might change as more genomes are included in the comparison.

In previous studies, widespread prevalence of ExPEC virulence-related genes have been reported in *mcr-1* carrying *E. coli* isolates (50, 51). Zhuge *et al.* (51) determined that almost half of the avian *mcr-1* positive *E. coli* (MCPEC) isolates showed pathogenicity in different animal models. Based on the criterion to identify an ExPEC pathotype, in this study, two isolates were defined as APEC with serotype O8:H4 and ST88. Apart from APEC virulence genes (*iut*A, *iss*, *hly*F, *iro*N, *omp*T) (52), these isolates also carried several ExPEC related virulence genes. ColV/ColBM plasmids were reported to be closely related with APEC/ExPEC pathogenesis and encoded virulence genes (*iucD*, *hlyF*, *etsC*, *ompTp*, *iroN*, *iutA*, *iss*, and *sitA*). It was worth noting that these two isolates carried ColVC plasmid replicon type. Moreover, Zhuge *et al*. (51) suggested that the zoonotic potential of MCRPEC is closely related to the ColV/ColBM type virulence plasmids, and poultry may be an important reservoir for human clinical infections. The rest of the isolates, although they harbor a wide range of virulence genes, it was not possible to assign to a certain group of human ExPEC, since the criteria to predefine ExPEC or its pathotypes were not applicable, due to the absence of one or two virulence gene.

Previously, Kürekci *et al.* (29) and Adıgüzel *et al*. (30) reported the detection of *mcr-1* in three and one *E. coli* strains isolated from retail raw chicken meat in Türkiye, respectively. The isolates from these studies were genetically distinct from our isolates as determined by both MLST (ST3941, ST1049, ST6094 vs ST10, ST88, ST156, ST1140) and whole genome-based phylogenetic tree (Fig. 3). Of these sequence types, *mcr-1* carrying *E. coli* ST10 clones have been reported to have worldwide distribution and is a major concern for human and animal health and should be kept under close surveillance within the One Health concept (27). ST88 has been previously reported in *mcr-1* positive pathogenic *E. coli* (53, 54), which carried genes related to enterotoxins such as *ast*A, *ltc*A, and *stb*. Indeed, the isolates belonging to ST88 in this study were positive *ast* gene encoding EAST1 toxin. Recently, an *mcr*-1 positive *E. coli* having multiresistant phenotype and genotype belonging to ST1140 recovered from pre-harvest broiler flocks have been reported in Lebanon (55). ST1140 was also shown to include two *mcr-1* positive *E. coli* with serotypes O38:H39 and O-:H39 from retail poultry meat in the Czech Republic and those reported in retail raw turkey meat originating in Poland, respectively (56). ST156 was detected in CMY-2 producing *E. coli* from Romanian poultry (57). Interestingly, following the first report of NDM-5- and

MCR-1-producing *E. coli* of ST156 in a muscovy duck (58); this clone was reported from fresh vegetables (59), human blood infection in Brazil (60), and healthy people in China (45). Furthermore, as it can be seen from phylogenetic analysis, the four previous Türkiye isolates were placed in three clusters together with Asian and European isolates of chicken, turkey, and migratory birds while the isolates from the current study were placed in four clusters and were closer to human, chicken and migratory bird isolates from Asia, Europe, Africa, and South America. Moreover, the phylogenetic analysis also indicated a close genomic relationship between *mcr-1* carrying *E. coli* isolates of human and animal origin in general, suggesting a potential transmission of *mcr-1* carrying isolates between these two settings. The tree also showed an overall high genetic diversity within *mcr-1* carrying isolates, indicating the transfer of this gene among bacterial hosts with different genetic backgrounds.

Following the first report of *mcr-1* carrying *E. coli* isolates from chicken and humans in 2016 in China, *mcr*-1 carrying *E. coli* isolates have been reported throughout the world in various sources. *mcr*-1 carrying *E. coli* from chicken meat samples was first reported by Kürekci *et al.* (29). In a multicenter center study conducted in 2017 in Türkiye, the presence of plasmid-mediated colistin resistance genes (*mcr*-1 and *mcr*-2) in human Enterobacterales was investigated, and was found that none of the isolates was positive for these genes. However, the first *mcr*-1 carrying *E. coli* was isolated by Özkaya *et al.* (61) from the urine culture of two patients over the age of 65 years who were under treatment in hospital. Arabaci *et al.* (62) detected the *mcr*-1 gene together with *bla*$_{\text{OXA-48}}$ in 3 carbapenem-resistant *Klebsiella pneumonia* isolates. These findings also further point out the everlasting spread of mobilizable colistin resistance mediated by *mcr* genes among Enterobacterales isolates with zoonotic potential in Türkiye.

In conclusion, the results of the study indicated that *mcr-1* carrying *E. coli* in broiler flocks in Türkiye was exist before the first report of *mcr*-1 positive *E. coli* in China in 2015. Therefore, the One-Health approach is required to manage mobilizable colistin resistance at different interfaces (human, animal, and environment).

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding authors.

Funding Support

There is no funding source.

Conflict of Interest

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

ÖA designed the study, conducted the experiments, analyzed the data, and wrote the manuscript. KB analyzed the data and wrote the manuscript. The authors contributed to the critical revision of the manuscript and have read and approved the final version.

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