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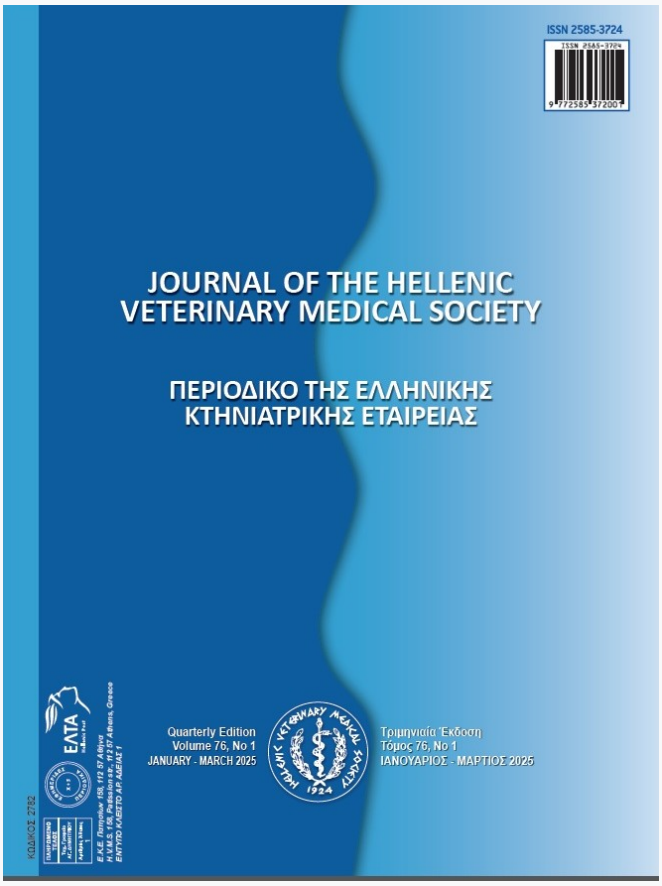

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Antimicrobial Susceptibilities, Its Resistance Mechanisms, Virulence Determinants and Phylogenetic Groups of *Escherichia coli* Isolated From Different Clinical Animal Samples

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ABSTRACT: *Escherichia coli* is a versatile agent, as some are commensals, normally living in the intestines of both humans and animals, while others are pathogenically responsible for a wide variety of intestinal and extra-intestinal infections including urinary tract infections (UTIs), meningitis, septicemia, bovine mastitis and colibacillosis in poultry. The present study aims to determine the antimicrobial susceptibilities, its underlying genetic resistance mechanisms, distribution of phylogroups, and virulence determinants of 42 *E. coli* isolates from different clinical sources of animals. Our results have revealed that phylogroup A1 (42.9%) and B1 (40.5%) were the most prevalent phylogenetic groups with different virulence profiles and varying incidence of virulence determinants. High rates of resistance to different categories of antimicrobial agents with high rate of MDR (59.5%). Phylogenetic analysis showcased remarkable diversity among the tested isolates, with no discernible clustering based on antimicrobial resistance or virulence patterns. Our research has demonstrated the significant phylogenetic diversity of *E. coli* isolated from different clinical samples. *E. coli* isolates are endowed with various virulence factors that contribute to their pathogenesis in animals. The elevated rates of antimicrobial resistance and emergence of MDR mirror the trend detected globally in recent years.

Key words: Antimicrobial Resistance; Clermont's phylogenetic typing; *Escherichia coli*; Virulence

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INTRODUCTION

Escherichia coli is a multifaceted resident of intestinal tract of human and animals. However, under certain conditions, some *E. coli* strains are able to pose a broad range of infections that lead a threat to human and animal health (Ramos et al., 2020). The prolonged and often indiscriminate use of antimicrobials has resulted in the selection and transmission of antimicrobial resistance in bacteria from different animal species (Caneschi et al. 2023). Exposure of the fecal microbiota to antimicrobials plays an crucial role by posing selective pressure on the microbiota, not only increasing the number of resistant bacteria and but also enabling the transmission of resistance genes to pathogenic bacteria (Serwecińska, 2020). Animals, humans and environment including water sources are natural habitats for virulent *E. coli* strains. Therefore, *E. coli* has been used as an indicator to monitor the emergence and levels of antimicrobial resistance in environments such as water sources, as well as in human and animal populations (Ramos et al., 2020). In previous studies, commensal *E. coli* isolates have been reported to be highly resistant to beta-lactams, cephalosporins (third generation and higher), fluoroquinolones, and aminoglycosides (O'Neill et al., 2023; Aslantaş, 2018), which are described as critically important antimicrobials by the World Health Organization (WHO, 2019).

Based on three genetic markers (*chuA*, *yjaA*, and, DNA fragment TSPE4.C2), *E. coli* strains were mainly divided into four phylogenetic groups (A, B1, B2, and D) (Clermont et al., 2000). Previous studies have consistently shown that commensal strains dwelled in phylogenetic groups A and B1, while the extra-intestinal strains fall into phylogenetic groups B2 and D (El-baz et al. 2022; Yılmaz and Aslantaş, 2019). Therefore, phylogenetic groupings of *E. coli* strains are valuable not only for defining *E. coli* communities but also for revealing the relation between phylotypes and diseases caused by the organism as well.

Pathogenic *E. coli* has an array of virulence determinants that play a significant role in its pathogenesis (El-baz et al. 2022; Yılmaz and Aslantaş, 2019). Moreover, it has been revealed that pathogenic strains have a superior prevalence of virulence traits than commensal ones (Sobhy et al., 2020). Based on their distinct virulence properties and host symptomatology, pathogenic *E. coli* strains are divided into two main groups: infection of the gastrointestinal system (Intestinal Pathogenic *Escherichia coli*, IPEC) or outside this (Extraintestinal Pathogenic *Escherichia coli*, ExPEC). IPEC strains are categorized into the following pathotypes: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC). Of these pathotypes, EIEC and EAEC strains are isolated only from humans. The ExPEC group includes the following pathotypes: uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), sepsis associated *E. coli* (SEPEC), avian pathogenic *E. coli* (APEC) and mammary pathogenic *E. coli* (MPEC) (Ramos et al., 2020). These virulence determinants include structural factors (fimbriae and flagella), iron-acquisition systems, secreted toxins, and capsules, which help the bacteria to (i) evade the host defenses, (ii) invade host tissues, and (iii) elicit local inflammation in the host (El-Shaer et al. 2018).

Among the structural factors, fimbrial adhesins plays an important role in adherence and colonization of the host epithelium, which include Type 1 fimbriae, P fimbriae, S fimbriae, and other fimbrial adhesins (Sobhy et al. 2020). To achieve sufficient level of iron, pathogenic *E. coli* use iron acquisition systems in response to iron limiting conditions inside the host (Rehman et al., 2017). A plethora of toxins are produced by different strains of *E. coli* that play different roles in their pathogenesis, such as heat labile (LTI and LTII), heat-stable (STa and STb), Shiga toxins (*stx1* and *stx2*), cytotoxic necrotizing factor (*cnf1* and *cnf2*), and hemolysin toxins (Ochoa et al., 2016). Virulence genes (VGs) found in pathogenic bacteria encode a variety of factors such as toxins, adhesins, and invasins, crucial for their pathogenicity. These genes are frequently situated on transmissible genetic elements known as pathogenicity islands (PAIs). However, it's essential to note that a commensal *E. coli* strain is not classified as pathogenic unless it acquires the requisite combination of virulence genes (Pakbin et al., 2021).

This study was conducted to determine VG genes, phylogenetic groups, antimicrobial susceptibility and genes mediating resistance in *E. coli* strains isolated from clinical cases in different animal species.

MATERIALS AND METHOD

Bacterial isolates

E. coli strains were isolated from different clinical specimens (internal organs, milk, diarrhea, swab) between June 2017 and November 2022 submitted

to Microbiology Laboratory for diagnostic purposes in Şanlıurfa and Hatay. Isolates were identified as *E. coli* based on classical biochemical tests (Procop et al., 2017), confirmed by polymerase chain reaction (PCR) targeting E. coli specific 16S rRNA (Yılmaz and Aslantaş 2019). All isolates were kept -20°C in Luria-Bertani medium containing 20% v/v glycerol until tests were performed.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of *E. coli* isolates were carried out by Kirby-Bauer disk diffusion method on Mueller-Hinton agar media in accordance with Clinical and Laboratory Standards Institute (CLSI, 2022) criteria. The following antibiotic disks (Bioanalyse, Türkiye) were used to define resistance profiles among *E. coli* clinical isolates: ampicillin (AM, 10 µg), amoxicillin-clavulanic acid (AMC, 10/20 µg), ceftazidime (CAZ, 30 µg), cefoxitin (FOX, 30 µg), cefepime (FEB, 30 µg), meropenem (MEM, 10 µg), gentamicin (CN, 10 µg), tobramycin (TOB, 10 µg), amikacin (AK, 10 µg), sulfametoxazole-trimethoprim (SXT, 1.25/23.75 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 5 µg) and chloramphenicol (C, 30 µg). *E. coli* ATCC 25922 was used a quality control strain. The isolates showing resistance to at least one antimicrobial belonging to three or more antimicrobial classes were defined as multidrug resistant (MDR) (Magiorakos et al., 2012).

DNA isolation

DNA extraction was made as previously described by Aslantaş (2018). Briefly, selected single colonies were grown in LB broth. An overnight culture (200 µl) was mixed with 800 µl of RNase/DNase free water and heated at 100°C for 10 min. Then, theresulting solution was centrifuged at 13 000 g for 10 min andthe supernatant was used as the DNA template and storedat -20°C until use.

Molecular detection of resistance genes

Resistance genes responsible for aminoglycoside (*aac(3)-IV*, *aadA*, *strA/B*, *aadB*, *aphA1*, and *aphA2*), trimethoprim-sulfamethoxazole (*sul1*, *sul2*, *sul3*, *dhfrI*, *dhfrIII*, *dhfrV*, *dhfrIX*, and *dhfrXIII*), chloramphenicol (*catI*, *catII*, and *catIII*), ceftazidime ($bla_{C\text{-}TX\text{-}M}$, bla_{SHV}, bla_{TEM}), tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG*), amoxicillin-clavulanic acid and cefoxitin ($bla_{CMY\text{-}2}$), and PMQR genes in ciprofloxacin-resistant isolates (*qnrA*, *qnrB*, *qnrC*, *qnrS*) were examined by PCR as previously reported by Aslantaş et al. (2023).

Molecular detection of virulence genes

The prevalence of virulence genes among *E. coli* isolates was screened by PCR as previously reported (Ewers et al. 2020; Yılmaz and Aslantaş, 2019) targeting adhesins: afimbrial adhesin (*afaD-8* and *afaE-8*), F17 fimbria (F17a-A, F17b-A, F17c-A, F17d-A), P (*papC* and *papE*) and S fimbria (*Sfa/focDE*), temperature sensitive hemagglutinin (tsh), iron acquisition systems: Iron repressible protein (*irp2*), aerobactin (*iucD*), yersiniabactin receptor (*fyuA),* toxins: Heat-stable enterotoxin STa, Heat-stable enterotoxin STb, Heat-labile enterotoxin LT, cytotoxic necrotizing factor 1-2 (*cnf-1, cnf-2*) Shiga toxin I-II (*stx-1*, *stx-2*), haemolysin (*hlyA*), intimin (*eaeA*) enteroaggragative stable toxin (EAST1), increased serum survival: increased serum survival protein (*iss*), vacuolating autotransporter toxin (*vat*), structural component of colicin V operon (*cva*), serum resistance (*traT*) and episomal outer membrane protease (*ompT*)

Diversity of *E. coli* Isolates using combined genotypic and phenotypic traits

A dendrogram was constructed utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), based on the presence or absence of virulence genes (VGs) and phenotypic resistance patterns. To analyze the data, a binary matrix was created scoring 1 or 0 according to the presence or absence of virulence genes and the susceptibility or resistance to antimicrobials, respectively. Then, the data was entered into the following site: http://insilico.ehu.es/dice_upgma/, dendrogram were plotted

RESULTS

Antimicrobial resistance phenotypes

Among the 42 *E. coli* strains, 34 (81%) showed resistance to at least one antimicrobial. The most common phenotypic resistances were to ampicillin (88.1%), tetracycline (34/42, 81%), ciprofloxacin (22/42, 52.4%), sulfamethoxazole/trimethoprim (19/42, 45.2%), amoxicyllin-clavulanic acid (15/42, 35.7%), chloramphenicol (15/42, 35.7%), ceftazidime (10/42, 23.8%), gentamicin (10/42, 23.8%. Lower resistance rates to tobramycin (5/42, 11.9%), amikacin (5/42, 11.9%), cefoxitin (4/42, 11.9%), and cefepime (3/42, 7.1%) were observed among the isolates. MDR phenotype was observed in 59.5% of the isolates. The most common antimicrobials implicated in MDR were ampicillin, tetracycline, ciprofloxacin, trimetho-

prim-sulphametexasole, and amoxicyllin-clavulanic acid. Six (14.3%) and four (9.5%) out of 42 isolates were designated as ESBL and pAmpC producer, respectively. Notably, all ESBL and pAmpC producers (except one isolate) were also MDR.

Molecular detection of virulence genes among *E. coli* clinical isolates

The prevalence of virulence genes among *E. coli* isolates was searched by PCR. Among adhesins, *papE* was detected in two (4.8%) isolates, *f17c-A* in one isolate (2.4%), *afaD-8* and *afaE-8* in two isolates (4.8%). Only 19% (n=9) of the isolates were negative for all virulence genes. None of the adhesins (*papC*, *Sfa/focDE, f17a-A*, *f17b-A*, *f111-A*) was detected among isolates. Iron acquisition systems were detected in *E. coli* clinical isolates, ferric yersiniabactin receptor (*fyuA*) was successfully amplified in 14 isolates (56.67%), *irp2* in 12 isolates and ferric aerobactin receptor (*iucD)* in 12 isolates (34.67%). The prevalence of the genes *traT* (in 22 isolates), *iss* (in 15 isolates), colV, *ompT* (in 14 isolates) (the episomal outer membrane protease that cleaves colicins) which are related to serum survival.

Phylogenetic classification

Isolates belonging to phylogenetic group A1 were found to be the most abundant in the collection (n=18; 42.9%). Twenty-eight percent (n=17, 40.5%) of the isolates were grouped in the B1 lineage. Five isolates were belonged to A0. No isolate was identified in B2, the phylogenetic lineage associated with virulent extraintestinal strains. Only one isolate was assigned to group D1, which is associated with pathogenic bacteria, although less frequently than group B2.

Occurrence of resistance determinants

The genotyping data correlated with the resistance phenotypes observed, with the identification of previously recognized marker genes in the majority of resistance cases. Tetracycline resistance, observed in 33 isolates of the isolates, was mediated dominantly by *tet*A, which was identified in 22 (66.7%) isolates. The *tetB* was identified in 5 isolates (15.2%), while two isolates (6.1%) were found to possess both *tetA* and *tetC*, one isolate (3.03%) carried *tetA* and *tetB*. Tetracycline resistance genes were not identified in three isolates classified to be resistant to this antibiotic. Mechanisms of chloramphenicol resistance were identified in 10 of the 15 resistant isolates. The *catI*, encoding chloramphenicol acetyltransferase, was only identified gene in these resistant isolates, whereas *catII* and *catIII* were not detected among resistant isolates. Mechanisms of trimethoprim-sulfometoxazole resistance were determined in 19 resistant isolates, and mediated by the *sul* and *dhfr* genes. While the sul genes were detected only in six isolates alone, the rest of the isolates carried both *sul* and *dhfr* genes together. Out of 10 gentamicin resistant isolates, four (40%) were positive for *aadB*, responsible for gentamicin resistance. The *aadB* gene was found together with aminoglycoside resistance determinants of *strA/B* and *aph1*. Genes encoding beta-lactamases were identified in 35 isolates, which was determined being resistant to at least one antimicrobial within this class. The bla_{TEM} gene was the most prevalent (n=33; 78.6%), followed by bla_{CTX-M} (n=7; 16.7%), bla_{CMY-2} (*n*= 4; 9.5%). No bla_{SHV} was detected among beta-lactam resistant isolates. Twenty and four isolates carried only bla_{TEM}, of which 20 was only resistant to ampicillin and four resistant to both ampicillin and amoxicillin-clavulanate. All bla_{CMY-2} positive isolates (n=4) were always accompanied by bla_{TEM}, and displayed resistance to ampicillin, amoxicillin-clavulanate, ceftazidime and cefoxitin. In contrast, out of 8 bla_{CTX-M} positive isolates, six isolates also carried bla_{TEM}, two isolates only possessed bla_{CTX} gene. Beta-lactam-susceptible isolates did not possess any of these markers.

Phylogroups of *E. coli* isolates

Phylogenetic analyses, integrating both genotypic and phenotypic traits, classified all isolates into four distinct clusters (Figure 1).

DISCUSSION

E. coli, a versatile organism, is responsible for a range of extraintestinal infections in both animals and humans. The treatment of such infections often necessitates the use of broad-spectrum antimicrobials including beta-lactams, cephalosporins, and fluoroquinolones. However, the extensive utilization of antimicrobial agents has exerted selective pressure, leading to the emergence of multidrug-resistant (MDR) organisms and facilitating the dissemination of resistance (Chinemerem et al., 2022). This has also led to animals becoming a potential reservoir of antimicrobial resistant bacteria (Caneschi et al., 2023). In the present study, *E. coli* strains isolated from different animal species were analyzed for antimicrobial susceptibilities, related acquired antimicrobial resistance genes, virulence genes and phylogenetic groups.

It has been reported that the ExPEC strains belon-

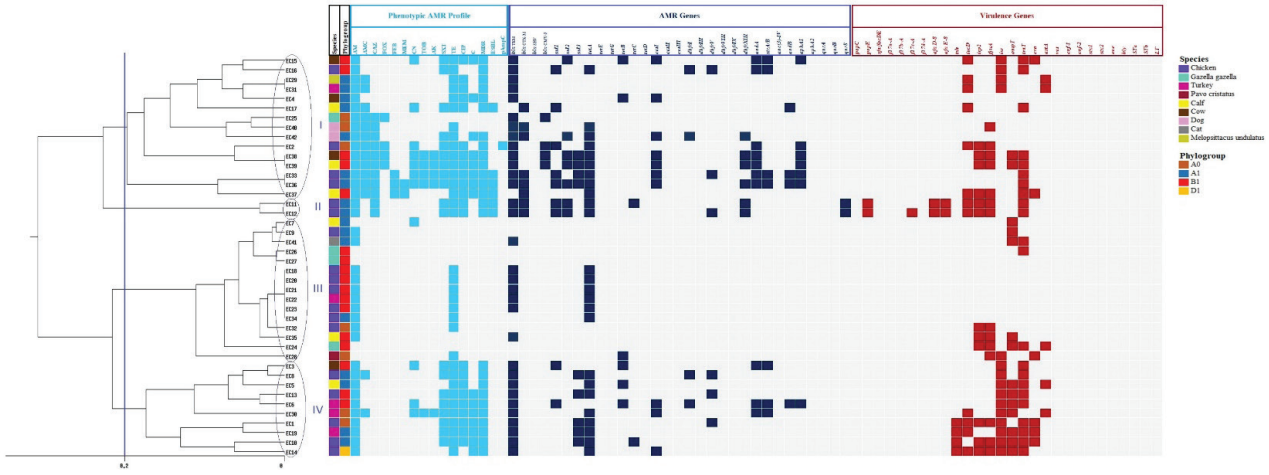

Figure 1. The dendrogram of *E. coli* strains drawn based on the presence or absence of VGs and phenotypic resistance patterns using unweighted-pair group method with arithmetic mean (UPGMA). AM: ampicillin; AMC: amoxicillin-clavulanic acid; CAZ: ceftazidim; FOX: cefoxitin; FEB: cefepime; MEM: meropenem; CN: gentamicin; TOB: tobramycin; AK: amikacin; SXT: sulfametoxazole-trimethoprim; TE: tetracycline; CIP; ciprofloxacin; C: chloramphenicol; MDR: multidrug resistance; ESBL: extended spectrum beta-lactamase; pAmpC: plasmid mediated AmpC resistance

ged to different phylogenetic groups, depending on their host origin (Clermont et al. 2000). Although human ExPEC isolates mainly belonged to one of two virulence groups (group B2 or D) and showed good correlation of phylogenetic grouping, animal ExPEC isolates can be phylogenetically distinct. Indeed, in this study, almost all isolates (97.6%) were determined to be in groups A and B1. Similarly, Maynard et al. (2004) and Karczmarczyk et al. (2011) reported that the majority of animal isolates belonged to phylogroups A and B1. Therefore, Karczmarczyk et al. (2011) reported that attention should be exercised when describing such bacteria as commensals. Hence, in this study, it is not feasible to assert that nearly all of the included isolates belong to phylogroups typically associated with commensal strains, nor is it appropriate to claim that these isolates accurately represent commensal strains prevalent in healthy animal.

Emergence and dissemination of antimicrobial resistance in clinical animal isolates represents one of the most important human- and animal health-threatening issues worldwide, which curb antimicrobial treatment options of bacterial infections. Therefore, continuous surveillance of antimicrobial resistance among both commensal and pathogenic bacteria have great importance to take actions so that mitigate antimicrobial resistance problem including better infection control, and greater conservation of existing agents. We found that more than 80% of the tested *E. coli* isolates were resistant to the common medically used antibiotics, such as tetracycline (81%), ciprofloxacin (52.4%), sulfamethoxazole/trimethoprim (45.2%), amoxicyllin-clavulanic acid (35.7%). In addition, 59.5% of the isolates displayed MDR phenotype. Higher rates of resistance have been recorded in extra-intestinal clinical isolates of *E. coli* from poultry and livestock in China (Yassin et al., 2017) and Nigeria (Aworh et al., 2021). High resistance rates might be explained by the widespread use of above-mentioned drugs to treat bacterial infections. These could have been associated with co-existence of resistance genes on same plasmid.

In this study, aminoglycoside resistance was observed in 11 isolates (26.2%) [Amikacin (11.9%), gentamicin (26.2%) and tobramycin (11.9%)]. The *aadB* gene, responsible for gentamicin and tobramycin resistance, was only detected in four (36.4%) isolates. This gene was accompanied by *strA/B* (conferring resistance to streptomycin) and *aph1* (conferring resistance to neomycin). This observation is consistent with previous studies showing that these genes are common in isolates resistant to other aminoglycoside drugs (Karczmarczyk et al., 2011).

The predominant mechanism of β-lactam resistance, particularly in Enterobacteriaceae, involves the production of β-lactamase enzymes. Continuous genetic mutations within β-lactam resistance encoding genes give rise to a diverse array of β-lactamase enzymes. Of notable significance are the extended-spectrum β-lactamases (ESBLs), which have garnered global attention and have been extensively reported, particularly among members of the Enterobacterales order. CTX-M, SHV, and TEM were the most com-

mon ESBL types reported worldwide. In addition, AmpC type beta-lactamases, conferring resistance to third generation cephalosporins, are major threat to healthcare worldwide (Khalifa et al., 2021). The most commonly detected β-lactamase gene in this study was bla_{TEM}, found 76.2% of *E. coli* isolates.

The prevalence of *bla*TEM is in good agreement with previous reports that found this gene to be widely disseminated among the *Enterobacteriaceae* from veterinary sources (Karczmarczyk et al., 2011; O'Keefe et al. 2010). None of the isolates carried bla_{SHV} gene. CTX-M β-lactamases are the dominant ESBL's in Enterobacterales in humans and farm animals (Zamudio et al. 2022). In this study, CTX-M type β-lactamase was detected in 8 (19%) isolates that displayed MDR phenotype as well. Negeri et al. (2023) reported that horizontal gene transfer (HGT) was a major contributor to widespread distribution of CTX-M-encoding genes, and a major factor in the co-transfer of genes encoding resistance to non-cephalosporins antimicrobials among bacterial populations. The bla_{CMY-2}, the most common plasmid-mediated AmpC β-lactamase in *E. coli*, was detected in 4 isolates, consistent with the genotype, all of these isolates exhibited resistance to amoxicillin-clavulanate and cefoxitin. Poultry is claimed to be a possible source of bla_{CMY-2} for humans (Börjesson et al., 2013). In a recent study, Aslantaş (2020) reported high occurrence of CMY-2-type beta-lactamase-producing *E. coli* among broiler flocks.

With the beginning of therapeutic use of tetracyclines in 1950s, this broad-spectrum antimicrobial agents began to be extensively used in the treatment of bacterial infections, and as growth promoters in animals. This has resulted in high rates of tetracycline resistance being reported among different bacterial species. Indeed, 78.6% of the isolates was resistant to tetracycline. Analysis of tetracycline resistant isolates by PCR revealed presence of *tetA*, *tetB* and *tetC* genes, with 66.7% of the isolates carrying *tetA*, 15.2% being positive for *tetB*. Two (6.1%) of these isolates had both *tetA* and *tetC*, and one isolate (3.03%) possessed both *tetA* and *tetB*. Tetracycline resistance genes identified in this study were associated with an efflux mechanism (Ng et al. 2001). In a previous study, *tetA* was reported as the most dominant tetracycline-resistant gene among *E. coli* strains isolated from animals (Karczmarczyk et al. 2011).

Bacterial resistance to sulfonamides primarily occurs through the acquisition of *sul1*, *sul2*, and *sul3* genes, which encode the dihydropteroate synthase enzyme. Conversely, resistance to trimethoprim is facilitated by the acquisition of *dhfr* genes, with over 30 variants reported to date, encoding the dihydrofolate reductase enzyme. Carriage of *su*l and *dhfr* genes on mobile genetic elements such as plasmids and integrons is closely associated with the emergence, evolution and spread of sulfamethoxazole-trimethoprim resistance (Whelan et al., 2023). In this study, *sul* (*sul1*, *sul2* and *sul3*) and dhfr genes (*dhfrI*, *dhfrV*, *dhfrXII*) were mostly detected together in sulfamethoxazole-trimethoprim resistant *E. coli* isolates, but *sul* genes were found alone in five isolates. This is consistent with previous studies reporting widespread distribution of sul and dhfr genes among *E. coli* isolates (Aslantaş, 2018; Karczmarczyk et al., 2011).

The *catI*, which encodes chloramphenicol acetyltransferase, was the only gene detected among chloramphenicol-resistant isolates. Despite the fact that use of chloramphenicol was banned in food-producing animals in Turkey (Regulation No: 2002/68 of 19 December 2002), 35.7% (15/42) of the isolates was resistant to chloramphenicol in the current study. This phenomenon could be elucidated by the persistence of chloramphenicol-resistant strains in the environment (Persoons et al., 2010) or by the coexistence of chloramphenicol resistance genes alongside other resistance genes on shared mobile genetic elements (Harada et al. 2006).

The main resistance mechanism against fluoroquinolones is mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* genes. In this study, plasmid-mediated quinolone resistance (PMQR) genes were investigated and only *qnrS* gene was detected in two-ciprofloxacin resistant isolates. Therefore, it can be suggested that the mechanism of fluoroquinolone resistance observed in the isolates is due to mutations in the QRDR of the *gyrA* and *parC* genes. Development of resistance to fluoroquinolones in commensal, pathogenic and zoonotic bacteria is of great concern since these antimicrobials are considered as clinically important drugs for human medicine (Aslantaş, 2018). High quinolone resistance (52.4%, 22/42) observed in this study could be attributed to misuse and overuse of these antimicrobials in both humans and animals in Türkiye for many years.

Most of the virulence genes examined in this study were selected for their association with *E. coli* strains causing extra-intestinal infections. In this study, 81% (34/42) of the isolates examined were positive at least for one virulence gene. In the present study,

a variety of adhesine genes including afimbrial adhesins (*afaD-8*, *afaE-8*), P fimbria (*papC* and *papE*), S fimbriae (*sfaS*/*focDE*), F17-related fimbria (F17a-A, F17b-A, F17c-A, F17d-A), intimin, and *temperature-sensitive hemagglutinin* (*tsh*) have been investigated. The expression of surface adhesins increases the virulence of pathogenic *E. coli* by starting close contact of the organism and the host cells. Of these, while *papE*, *afaD-8* and *afaE-8* were found in only two chicken isolates together, these adhesins were not found in the remaining of the isolates. The *f17c-A* was only F17 related fimbriae, which was detected in only one chicken isolates along with *papE*, *afaD-8* and *afaE-8*. None of the isolates contained *eae* gene encoding intimin. The temperature-sensitive hemagglutinin (*tsh*) is an autotransporter protein secreted by avian-pathogenic *Escherichia coli* strains that colonize the respiratory tract and lead to airsacculitis, pericarditis, and colisepticemia (Kostakioti and Stathopoulos, 2004). This gene was identified in 3 chicken and one turkey isolates.

The heat-stable (ST) and heat-labile (LT) enterotoxins are important virulence factors in ETEC. Both toxins induce secretion of water and ions resulting in watery diarrhea. Epidemiological studies imply that strains producing ST and/or LT elicit the most severe diarrhea among children (Wang et al. 2019) and cattle and buffalo calves (Awad et al. 2020). These toxins were not found among the isolates. However, enteroaggragative stable toxin (EAST1) was detected in 11.9% (5/42) of the tested isolates (2 turkey, 1 calf, 1 *Gazella gazella*, 1 Melopsittacus undulatus). EAST1 has been rarely associated with cases of diarrhea in animals and humans (Dubreuil, 2019). Colicin V plasmid operon genes, *cvaA/B* was detected in six (14.3%) isolates. The *cvaA/B* is one of the crucial factors that contribute to the virulence in APEC, and involved in different combinations of virulence genes to predict disease-causing potential of APEC strains (Joseph et al. 2023). The cytotoxic necrotizing factor 1 and 2 (CNF1-2) are a Rho GTPase protein toxin that promotes invasion into host cells. It is rarely detected in the stool of children with diarrhea, but is more common among ExPEC, including urinary tract infections (UTIs), bacteriaemia, and meningitis in neonates (Fabbri et al. 2010). Açık et al. (2004) reported that 6.6% and 16.4% of *E. coli* strains isolated from cow and sheep milk samples were positive for *cnf2* gene alone and in combination with *traT* gene, respectively. The vacuolating autotransporter toxin (*vat*) belongs to class II serine protease AT protein of Enterobacterales, which is cytotoxic to chicken embryonic fibroblasts and plays a role in avian cellulitis infection (Nichols et al., 2016). The *vat* gene was absent in all of the tested *E. coli* clinical isolates. The pathogenicity of the Shiga toxin-producing *E. coli* (STEC) pathotype is primarily linked to the production of Shiga toxins 1 and 2 (*stx1* and *stx2*). While STEC commonly inhabit the intestines of healthy cows, this pathotype is notorious for causing severe clinical manifestations in humans. These can include hemorrhagic colitis, hemolytic uremic syndrome, and various other severe disease conditions (Awad et al. 2020). The pathogenicity of the STEC pathotype is attributed to the production of Shiga toxins 1 and 2 (*stx1* and *stx2*). Although STEC are harbored in the intestines of healthy cows, this pathotype is associated with severe clinical signs in humans characterized by hemorrhagic colitis, hemolytic uremic syndrome and other severe disease conditions. Cengiz and Adıgüzel (2020) detected at low rates of *stx1* (2.3%, 3/133) and *stx2* (3.8%, 5/133) genes in *E. coli* isolated from calves with diarrhea. In contrast, Kızıl et al. (2024) reported higher prevalence rates for *stx1* (45.9%) and *stx2* (64%) in *E. coli* isolates from sheep, goat and cattle. However, *stx1* and *stx2* were not detected in the isolates.

Iron is a vital element for all bacteria and catalyzes a wide range of vital enzymatic reactions; however, it is crucial to the host cells as well. PCR amplification of three siderophore genes among 42 *E. coli* isolates revealed that *iucD*, *fyuA* and *irp2* genes were present among 12 (28.6%), 14 (33.33%) and 12 (28.6%). Furthermore, the combination *fyuA, irp2*, and *iucD* was mostly present in APEC in accordance to Paixão et al. (2016).

The bacterial ability to survive in the blood has been attributed to resist or counteract from immune mechanisms of the host by polysaccharide capsule, surface proteins, lipopolysaccharide (LPS) (Miajlovic and Smith, 2014). In this study, 64.3% (27/42) of the isolates carried at least one of serum survival proteins alone or in combination. The frequencies of genes related with resistance to serum, *traT*, *iss* and *ompT* were detected as 52.4%, 35.7% and 33.3%, respectively. Of these, *traT* was the most common serum resistance gene, which encodes an outer lipoprotein interacting with the complement system. Although high prevalence of *traT* gene among *E. coli* isolated from dairy cows with clinical mastitis has been reported, the role of the *traT* gene as a primary virulence mechanism of *E. coli* in mammary infections is contro-

versial (Guerra et al., 2020). The *ompT* and iss genes have been reported to be widespread among APEC isolates, and being one of the important virulence factors enhancing pathogenicity of APEC strains (Azam et al., 2020).

CONCLUSIONS

The limited number of isolates utilized in this study constrains our ability to draw highly specific conclusions from the comparison among animal clinical isolates. However, despite this limitation, some general observations can still be gleaned from our data. For instance, ExPEC isolates, both within and across animal and human groups, may exhibit relatively distinct profiles. This observation implies that the assortment and diversity of genes contributing to phenotypic resistance are subject to dynamic evolution, shaped by selective pressures exerted by antimicrobial usage. In addition, our results revealed that virulence factors contributing to *E. coli* pathogenesis are distributed among different phylotypes. The high rates of antimicrobial resistance also pose a serious health challenge for the one-health approach and limit the therapeutic options available for the treatment of infections caused by pathogenic *E. coli* isolates.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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