Original Research Article

EMERGENCE OF COLISTIN RESISTANCE IN EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING *ESCHERICHIA COLI* ISOLATES FROM DAIRY COWS IN TÜRKİYE

ABSTRACT

Background: In recent years, the reporting of extended-spectrum beta-lactamase producing and colistin-resistant *Escherichia coli (E. coli)* in food-producing livestock animals is of great importance as a potential vector of multi-drug resistant (MDR) *E. coli* for the farm environment, farm workers, and veterinarians who are in close contact with these animals. In this study, it was aimed to determine antibiotic resistance profiles of *E. coli* from diarrhoeic dairy cows, and observe the resistance against beta-lactam group antibiotics and colistin in 3 years' time.

Materials and Methods: For the isolation, 1g of sample was suspended into 9 mL of modified Tryptone Soya Broth and incubated overnight at 37 °C. After the incubation, the broth was inoculated on MacConkey Agar and EMB Agar and incubated aerobically at 37 °C for 24h. Identification was done according to biochemical tests. *E. coli* isolated from the fecal samples of diarrhoeic dairy cows were investigated for the antibiotic resistance with the six different antibiotic classes by Kirby Bauer Disc Diffusion Test.

Results: Fifty-four *E. coli* isolated from the fecal samples of diarrhoeic dairy cows were investigated for the multi-drug resistance. Fourty-four *E. coli* showed MDR resistance, including colistin; nine *E. coli* isolates showed MDR resistance profiles involving at least six to ten antibiotics , but not for colistin. It was discovered that MDR *E. coli*

isolates were also resistant to colistin. Resistance to beta-lactams were observed 100% in 2021 and 2022, but not for 2020. Colistin resistance was found to have increased progressively year over year, reaching 0% in 2000 and 37.03% and 46.29% in the next two years, respectively.

Conclusion: As a conclusion, circulation of beta-lactamase producing *E. coli* accompanied with colistin resistant *E. coli* in live-stock animals reared for the purpose of milk production should be concerned as a potential public health problem in terms of one health concept and more detailed investigations should be planned to question the ground origins of gradual increase.

*Keywords:*beta-lactamase, colistin, resistance, *E. coli*, bovine feces

1.INTRODUCTION

Escherichia coli is a common causative agent of enteric foodborne ilness, urinary tract infections, blood stream infections, a and gastroenteritis in animals and humans worldwide [1]. The emergence of multidrug resistant (MDR) Enterobactericeae including *E. coli* has become a major concern throughout the world and limited availability of novel antibiotics, colistin usage has been resumed as a last resort antibiotics in human and veterinary medicine [2].

Since the 1950s, colistin has been used for decades in veterinary medicine to treat and prevent infectious diseases, especially gastrointestinal infections caused by Gram-negative bacteria in systems of intensive husbandry [3]. The gradual increase of colistin-resistant *E. coli* prevalance in foods and food-producing animals have great

importance for human health since colistin, a last-resort antibiotics used for the human potentially fatal infection treatment by multiresistant-enterobacteria [4]. Since resistance to antibiotics like extended-spectrum cephalosporins (ESCs), carbapenems, and colistin has spread widely in humans, animals, and the environment, antimicrobial resistance has grown to be a serious public health concern [1]. The extended-spectrum cephalosporins are among the beta-lactam antibiotics to which *Enterobactericeae* develop resistance through a variety of molecular strategies. The production of extended spectrum beta-lactamases (ESBLs) is the primary mechanism by which resistance to these antibiotics is induced [5]. The main types of ESBLs identified in species belonging to the Enterobacterales family are Temoneira (TEM), Sulfhydryl Variable (SHV), and Cefotaximase-Munich (CTX-M). CTX-M, TEM, and SHV-type beta-lactamases have been identified as major ESBL genes (blaTEM, blaSHV, blaCTX-M) in plasmid-associated carbapenem-resistant Enterobacteriaceae [6]. Colistin resistance in gram negative bacteria is generally divided into two mechanisms: plasmid mediated and chromosomal mediated [1]. Apart from the chromosomally-mediated mechanisms, 10 variants, *mcr-1* to *mcr-10*, carried by various plasmid families have been so far identified in *Enterobacterales*, especially in *E. coli* and *Enterobacter* spp. [6]. Plasmid-mediated resistance to ESCs and colistin in animals raised for food production has gained attention in recent years. [7]. Given need to retain the efficacy of antimicrobials used to treat MDR infections in humans, the use of colistin in veterinary medicine is re-evaluated. Numerous studies on chicken and chicken meat have also been carried out, and reports of a significant worldwide prevalence of ESC-resistant and colistin resistant *E. coli* have been made [8, 9]. Contrarily, there is a lack of research conducted on

ruminants. In France and the Netherlands, the range of rate between 20.4-39.0% ESC-R *E. coli* harboring mcr-1 gene was reported [10,11].

In recent years, the coexistence of colistin resistance with ESBL-resistant *E. coli* of both human and veterinary origin, and the circulation of common resistance patterns between these two populations, has raised questions about the use of colistin, considered an antibiotic of last resort, in the veterinary field as well. The aims of this study were to determine antimicrobial resistance profiles of *E. coli* from diarrhoeic dairy cows, screen and evaluate for resistance to beta-lactam antibiotics and colistin resistance between 2020-2022.

2.MATERIAL AND METHODS

2.1 Fecal Samples

Between 2020 and 2022, 54 fecal samples were collected from the same dairy cow farm in Bandirma, Türkiye. To get fresh stools, the faeces were collected with the assistance of a veterinarian. A steril wooden medical spatula were used to collect 10 gr fecal sample per animal. The veterinarian reported that animals in the farm were under standart rules of hygiene, food consumption and restricted antibiotic use. Samples were collected using sterile tubes and sterile spatula. The samples were transferrred to laboratory in two hours, immediately under cold chain.

2.2 Isolation and identification of *E. coli*:

Detection of *E. coli* was carried out according to the protocol of Quin et al. [12]. Approximately 1g of sample was suspended into 9 mL of modified Tryptone Soya Broth (Oxoid, CM0129) and incubated overnight at 37 °C. After the incubation, the

broth was inoculated on MacConkey Agar (Oxoid, CM0115.) and incubated aerobically at 37 °C for 24h. The suspected colonies with round shapes, smooth surfaces and pink color on MacConkey Agar were inoculated on Eosin-Methylene Blue (EMB) Agar (Oxoid, CM0069). At the end of incubation, the suspected colonies of *E. coli* for the characteristic metallic sheen on Agar were examined with Gram's stain. After the examination, one *E. coli* colony was inoculated into Brain Heat Infusion Broth (BHIB) (Oxoid, CM1135) Pure culture of *E. col*i were identified on biochemical tests, oxidase test, coagulase test, catalase test, indole test, methyl red test, Voges-Proskauer test, citrate (Simmons) test [13]. Afterwards, the cultured samples were stored at -20 °C for antibiotic susceptibility testing.

2.3 Antimicrobial susceptibility testing:

For antimicrobial susceptibility testing, a total of 11 antibiotic discs (Oxoid) belonging to six different antibiotic classes- enrofloxacin (ENR 5µg), gentamicin (GEN 10µg), amoxillin/cluvalanic acid (AMC 20/10µg), trimethoprim-sulfamethoxazole (SXT 25µg), oxytetracycline (OT 30µg), cephaperazone (CFP 75µg), neomycin (N 30µg), cefquinom (CEQ 30µg), cephalexin/kanamycin (CFXK 15 µg), tetracyclin (TE 30 µg), colistin (CT 1 µg)- were used in Kirby-Bauer Disc Diffusion Method. The zone diameters were interpreted according to European Committee on Antimicrobial Susceptibility Testing Standarts (EUCAST) [14], and evaluated as sensitive, intermediate and resistant.

3.RESULTS

In our study, all the samples were collected from the cow farm in Bandirma-Türkiye. Between 2000- 2022, 41 out of 54 *E. coli* isolated from dairy cows which suffered diare were found to be haemolytic, the remaining 13 were non-haemolytic. In order to cure enteritis the antibiotics emphasized in Table 1 were used.

Between 2020 and 2022, phenotypically nine and 44 out of 54 were determined to be moderate sensitive and resistant to colistin, respectively. Three out of 13 non-haemolytic *E. coli* were determined to be colistin resistant, the remains were moderate sensitive while all haemolytic *E. coli* exhibited colistin resistance. When the rates of colistin resistance were evaluated over the three years, the rate, which was found to be 0% in the year 2020, increased to 37.03% and 46.29% in the subsequent two years, respectively. Except for the year 2020, in the other two years, 100% resistance to beta-lactam group antibiotics was exhibited, and parallel to this, it was observed that resistance was demonstrated in all 20 *E. coli* isolates except for five isolates in 2021, and in all 25 isolates in 2022 (Table 1).

E. coli isolates from dairy cows with diare displayed multi-drug resistance to fluoroquinolone (ENR), aminoglycoside (GEN and N), beta-lactam (AMC, CFP, CEQ, CFXK), tetracycline (TE and OT), sulfonamide (SXT) group antibiotics. MDR profiles *E. coli* were emphasized in detail in Table 2.

Nine *E. coli* isolates exhibited MDR resistance profiles at least six to ten antibiotics, but not for colistin, 44 *E. coli* exhibited MDR resistance including colistin. Colistin resistant *E. coli* isolates were found to be MDR, as well (Table 2).

When we evaluated resistance to beta lactam group antibiotics; in 2020 none of the *E. coli* isolates exhibited resistance against colistin while AMC/CFP/CEQ/CFXK, CFP/CFXK, CFCK and CEQ resistance profiles were observed in each one among four *E. coli* isolates, respectively. In 2021, AMC/CFP/CEQ/CFXK resistance profiles were observed in five *E. coli* isolates however those were sensitive to colistin. In the same year and following year, AMC/CFP/CEQ/CFXK/CT resistant profiles were determined in 20 and 25 isolates, respectively. An increase in resistance to colistin has been observed in parallel with the year-by-year increase in resistance to beta-lactam group antibiotics (Table 3).

4. DISCUSSION AND CONCLUSIONS

There is a dearth of information on the colistin resistance of animal microorganisms and foodstuffs. The European surveillance of bacteria of animal origin did not include colistin in its mandatory antimicrobial panel for *Enterobacteriaceae* until 2014 [15]. Similarly, polypeptides were not relatively considered in the antimicrobial resistance monitoring schemes of many other countries. This condition can be explained by several factors. First, the exclusion of polypeptides from epidemiological studies and animal-origin bacterial monitoring systems in many nations likely stemmed from the lack of colistin usage or its restricted application in both humans and animals in these regions. Subsequently, scientists and veterinarians likely assumed that colistin resistance was rare due to co-selection of colistin resistance by other antibiotics or clonal expansion of a colistin-resistant (CST-R) isolate because colistin resistance was thought to be limited in *Enterobacteriaceae* (which are the main indicators of resistance in Gram-negative bacteria). It was thought to solely been caused by chromosomal

mutations until the end of 2015 [16]. Ultimately, they neglected colistin resistance surveillance. However, the use of colistin as a last-resort antibiotic in people has increased, making it necessary to monitor the development of resistance to this polypeptide in humans, companion animals, and food animals with more precision and thoroughness.

In veterinary field, among farm animals such as pigs, ruminants, poultry and companion animals such as rabbits, horses, dogs, cats colistin usage has been widely-spreaded in all continents for decades [17]. Isolated CST-R *E. coli* in pigs, hens, broilers, ducks, rabits from France [18], in companion animals such as horses, dogs, cats from Sweden [19], Norway [20], Germany [21], Türkiye [22], in piglets from Thailand [23], in yaks from India [24], in diarrhoeic ruminants from Spain [25] were reported among the animals including companion animals and live-stock animals.

In Türkiye, the studies conducted on colistin resistance was limited and studied group comprised animals and poultry [26-28]. Erzaim [26] declared that fifteen out of 200 *E. coli* isolated from the intestine of broiler chickens was phenotypically resistant to Polimiksin E and none of colistin resistance were found to be plasmid-mediated. Sezener et al [27] reported 22.66% colistin resistance among 75 *E. coli* isolated from cats and dogs, and none of the isolates were found to harbor mcr 1-3 gen. When Tok [28] investigated *E. coli* and *Salmonella* isolates the author declared that *E. coli* and *Salmonella* isolates collected from chicken meat samples between 2018-2019 showed evidence of CST-R with the rate of 25% and 53%, respectively while historical *E. coli* and *Salmonella* isolates exhibited 0% and 8%, respectively. According to the results of Tok [28], colistinresistance was seen to be increased over time among both *Salmonella*

and *E*. coli isolates. Parallel to the previous study the rate of colistin resistance in the current study was found to be gradually increased over the years. When the rates of colistin resistance were evaluated over the three years, the rate, which was found to be 0% in the year 2000, increased to 37.03% and 46.29% in the subsequent two years, respectively. The inappropriate use of colistin in agriculture as a growth promoter in livestock has contributed to the selection of resistant strains. Inadequate infection control practices, poor sanitation, mobility of people and animals have also contributed to the spread of CST-R *E. coli* in the dairy farm [29].

High MDR resistance occured in *E. coli* isolates in the current study to fluoroquinolone (ENR), aminoglycoside (CN and N), tetracycline (TE and OT), sulfonamide (SXT) group, polymxine (colistine sulphate) antibiotics were thought not to be astonishing. In alignment with our study, MDR *E. coli* from the feces of bovine animals in Europa was declared [30]. Sezener et al. [27] also reported multi-drug resistance to similar antibiotics in *E. coli* isolates from cats and dogs in Türkiye. Recently, global increase in human *E. coli* isolates exhibiting resistance to beta lactams, cephalosporins possesing bla_{CTX}, bla_{NDM} , bla_{TEM} were reported to be resistant to colistin harboring plasmid mediated *mcr* genes, as well food-producing farm animals in Latin America, Europea, and Asia [30, 31, 32]. In our study, the same situation was observed in a dairy cow farm. Except for the year 2020, along with 100% resistance to beta-lactam group antibiotics in the other two years, colistin resistance was demonstrated in all 20 *E. coli* isolates except for five isolates in 2021, and in all 25 isolates in 2022 (Table 1). *E. coli* can be found in the intestines of animals and humans, and food. CST-R *E. coli* in livestock can contaminate meat and milk; should they are not handled or cooked

properly, great risks to human health can occur [33]. Recent studies showed that ESBLs and carbapenem resistance accompanied emerging CST resistance in the same bacteria have great significance in terms of health asssociated MDR infections [1]. In our study, beta lactam group-AMC, CFXK and ESBL group- CFP, CEQ- resistant *E. coli* isolated from the feces of diarrhoeic dairy were also found to be resistant to colistin. Supporting our results, it has been demonstrated that ESBL-producing *E. coli* can acquire colistin resistance in vitro experiments by Nakayama et al [34]. In parallel to our results, in a study conducted in Tunisia, Saidani et al. [35] declared where they detected 14 ESBL-producing *E. coli* in 219 fecal samples, they found that 11 of those also exhibited colistin resistance. Similiarly, in France of 106 out of 517 ESBL-producing *E. coli* isolates carrying mcr-1 gene were also found to be resistant to colistin [36]. Moreover, Mcr-1gene carried on IncHI2 plasmid in CST-R *E. coli* from calf fecal samples in Tunisia between 2016-2017 and bovine digestive samples in France between 2004-2014 [37] were reported. Those were co-localized with $bla_{\text{CTX-M-14}}$, blaCTX$_{\text{-M-55}}$ and $bla_{\text{CTX-M-14}}$, $_{\text{blaCTX-M-1}}$ in isolated ESBL producing *E. coli* [35]. Although we did not examine the presence of both mcr gand bla_{CTX} genes in our isolates, colistin resistant *E. coli* exhibiting resitance to beta lactam group antbiotic AMC; CFXK (2. Generation cephalosporin); and ESBL group antibiotics, CFP (3. Generation sephaloporin); CEQ (4. Generation cephalosporin) were suspected to harbor those genes. In further studies, the presence of those will be investigated in detail in our isolates. The study's findings all point to the necessity of a thorough, one health strategy that prioritizes cleanliness, prudent use of antibiotics in agriculture, and antibiotic stewardship in clinical settings [38].

Consequently, the increasing colistin resistance exhibited by ESBL-resistant *E. coli* in food-producing farm animals from year to year, along with the similar resistance patterns observed in human *E. coli* isolates globally poses a significant public health threat. Although our study focused on phenotypic resistance and did not examine the transfer of common resistance genes, future research should specifically investigate similar resistance genes in live-stock animals, as well as the spread of epidemic plasmids in both humans and animals. As a result, concerted efforts are required in both human and veterinary medicine.

ETHİCAL APPROVAL

According to the subparagraph k of the 8th article of the "Regulation on the Principles and Procedures of Animal Experiment Ethics Committees" published in the Official Gazette dated 15.02.2014 and numbered 28914, the collection of fecal or bedding samples and sample collection by swabbing are not subject to the approval of the Local Ethics Committee for Animal Experiments (HAYDEK).

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Zhang S, Abbas M, Rehman MU, Wang M, Jia R, Chen S, Cheng A, Liu M, Zhu D, Zhao X, Gao Q, Tian B, Cheng A. Updates on the global dissemination of colistin-

resistant *Escherichia coli*: An emerging threat to public health. Sci Total Environ. 2021; 799:149280.

DOI: 10.1016/j.scitotenv.2021.149280.

2. Pogue JM, Jones RN, Bradley JS, Andes DR, Bhavnani SM, Drusano GL, Dudley MN, Flamm RK, Rodvold KA, Ambrose PG. Polymyxin susceptibility testing and interpretive breakpoints: recommendations from the United States Committee on Antimicrobial Susceptibility Testing (USCAST). Antimicrob Agents Chemother 2020; 64(2): e01495-19.

DOI: 10.1128/AAC.01495-19.

3. Jansen W, van Hout J, Wiegel J, Iatridou D, Chantziaras I, De Briyne N. Colistin use in european livestock: Veterinary field data on trends and perspectives for further reduction. Vet Sci. 2022; 9(11): 650.

DOI: 10.3390/vetsci9110650.

4. World Health Organization: Resistencia a Los Antimicrobianos. Accessed 05.September 2024. Available:http://www.who.int/es/news-room/fact-sheets/detail/antimicrobial-resistance.

5. Landolsi S, Selmi R, Hadjadj L, Ben Haj Yahia A, Ben Romdhane K, Messadi L, Rolain JM. First report of extended-spectrum β-lactamase (bla CTX-M1) and colistin resistance gene mcr-1 in E. coli of lineage ST648 from cockroaches in Tunisia. Microbiol Spectr. 2022; 10(2): e0003621.

6. Li, M.; Guo, M.; Chen, L.; Zhu, C.; Xiao, Y.; Li, P.; Guo, H.; Chen, L.; Zhang, W.; Du, H. Isolation and Characterization of Novel Lytic Bacteriophages Infecting

Epidemic Carbapenem-Resistant Klebsiella pneumoniae Strains. Front. Microbiol. 2020, 11, 1554

DOI: 10.1128/spectrum.00036-21

7. Shafiq M, Rahman SU, Bilal H, Ullah A, Noman SM, Zeng M, Yuan Y, Xie Q, Li X, Jiao X. Incidence and molecular characterization of ESBL-producing and colistin-resistant Escherichia coli isolates recovered from healthy food-producing animals in Pakistan. J Appl Microbiol. 2022; 133(3):1169-1182.
DOI: 10.1111/jam.15469.
8. Lemlem M, Aklilu E, Mohammed M, Kamaruzzaman F, Zakaria Z, Harun A, Devan, SS. Molecular detection and antimicrobial resistance profiles of Extended-Spectrum Beta-Lactamase (ESBL) producing Escherichia coli in broiler chicken farms in Malaysia. PLoS One. 2023; 18(5): e0285743.
DOI: 10.1371/journal.pone.0285743.
9. Chileshe C, Shawa M, Phiri N, Ndebe J, Khumalo CS, Nakajima C, Kajihara M, Higashi H, Sawa H, Suzuki Y, Muleya W, Hang'ombe BM. Detection of extended-spectrum beta-lactamase (ESBL)-producing enterobacteriaceae from diseased broiler chickens in Lusaka District, Zambia. Antibiotics (Basel) .2024; 13(3): 259.
DOI: 10.3390/antibiotics13030259.
10. Hordijk J, Wagenaar JA, van de Giessen A, Dierikx C, van Essen-Zandbergen A, Veldman K, Kant A, Mevius D. Increasing prevalence and diversity of ESBL/AmpC-type βlactamase genes in *Escherichia coli* isolated from veal calves from 1997 to 2010. J Antimicrob Chemother. 2013; 68(9): 1970-1973.

DOI: 10.1093/jac/dkt132.

11. Gay E, Bour M, Cazeau G, Jarrige N, Martineau C, Madec J-Y, Haenni M. Antimicrobial usages and antimicrobial resistance in commensal *Escherichia coli* from veal calves in France: evolution during the fattening process. Front Microbiol. 2019; 10:792.

    DOI: 10.3389/fmicb.2019.00792.

12. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. London, United Kingdom: Wolf/Mosby Press; 1994.

13. Holt JG, Krieg NR, Senath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. 9th ed. Baltimore Md, United States: Willaims and Wilkins; 1994.

14. European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters. Accessed 05.September 2024. Available:http:http://www.eucast.org .

15. 2013/652/EU. Commission Implementing Decision on the Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal Bacteria. Official Journal of the European Union. 2013.

16. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016; 16(2):161-168.

    DOI: 10.1016/ S1473-3099(15)00424-7.

17. Kempf I, Jouy E, Chauvin C. Colistin use and colistin resistance in bacteria from animals. Int J Antimicrob Agents. 2016; 48(6): 598-606.
DOI: 10.1016/j.ijantimicag.2016.09.016.
18. Jarrige N, Jouy E, Haenni M, Gay E, Madec JY. Résapath: Réseau d'épidémiosurveillance de l'antibiorésistance des bactéries pathogènes animales. Bilan 2014. ANSES. Accessed 05.September 2024. Available: https://www.anses.fr/fr/system/files/LABO-Ra-Resapath2014.pdf
19. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Accessed 05.September 2024.
Available: https://www.folkhalsomyndigheten.se/pagefiles/20281/Swedres-Svarm201414027.pdf
20. Use of antimicrobial agents and occurrence of resistance to antimicrobial agents in Norway. Accessed 05.September 2024. Available: http://www.vetinst.no.
21. Goncagul G, Seyrek Intas K. Antimicrobial susceptibility of bacteria isolated from uteri of thoroughbred mares with fertility problems in Germany. Kafkas Univ Vet Fak Derg. 2013; 19(7): 105-109.
DOI : 10.9775/kvfd.2012.8094.
22. Goncagul G, Gocmen, H, Yendim SK, Yilmaz K, & Intas KS Bacteriologic and cytologic examination results of mares with pneumovagina in Bursa region. Int J Vet Sci. 2016; 5(4): 295-298.
23. Prapasarakul N, Tummaruk P, Niyomtum W, Tripipat T, Serichantalergs O. Virulence genes and antimicrobial susceptibilities of hemolytic and nonhemolytic

Escherichia coli isolated from post-weaning piglets in central Thailand. J Vet Med Sci. 2010; 72(12):1603-1608.

DOI: 10.1292/jvms.10-0124.

24. Bandyopadhyay S, Lodh C, Sarkar M, Ghosh MK, Bera AK, Bhattacharyya D, Mondal DK, Baruah KK. Prevalence, molecular fingerprinting and drug resistance profile of enterovirulent Escherichia coli isolates from free-ranging yaks of Tawang District, Arunachal Pradesh, India. Trop Anim Health Prod. 2012; 44(5):1063-1072.

DOI: 10.1007/s11250-011-0041-9.

25. Medina A, Horcajo P, Jurado S, De la Fuente R, Ruiz-Santa-Quiteria JA, Dominguez-Bernal G, Orden JA. Phenotypic and genotypic characterization of antimicrobial resistance in enterohemorrhagic Escherichia coli and atypical enteropathogenic E. coli strains from ruminants. J Vet Diagn Invest. 2011; 23(1): 91-95.

DOI: 10.1177/104063871102300114.

26. Erzaim N. Investigation of the plasmid mediated colistin resistance gene mcr-1 in *Escherichia coli* isolates from chicken feces. Master of Science Thesis, Türkiye: İstanbul University Cerrahpasa, Institute of Graduate Studies. 2018.

27. Sezener MG, Findik A, Ergüden VE, Gülhan T, Çiftci A, Boynukara B. Colistin Resistance Profiles and Genotypes of *Escherichia coli* Isolates from Dogs and Cats. Acta Scientiae Veterinariae. 2022; 50: 1894.

DOI: 10.22456/1679-9216.126741.

28. Tok S. Investigation of plasmid mediated colistin resistance phenotypically and genotypically in *Salmonella* and *E. coli* isolates in Turkey. Türkiye Middle East Technical University. Master's Thesis. 2022.
29. Bastidas-Caldes C, de Waard JH, Salgado MS, Villacís MJ, Coral-Almeida M, Yamamoto Y, Calvopiña M. Worldwide prevalence of mcr-mediated colistin-resistance *Escherichia coli* in isolates of clinical samples, healthy humans, and livestock-A systematic review and meta-analysis. Pathogens. 2022; 11(6): 659. DOI: 10.3390/pathogens11060659.
30. Brennan E, Martins M, McCusker MP, Wang J, Alves BM, Hurley D, Garch FE, Woehrlé F, Miossec C, McGrath L, Srikumar S, Wall P, Fanning S. Multidrug-resistant *Escherichia coli* in bovine animals, Europe. Emerg Infect Dis. 2016; 22(9):1650-1652. DOI: 10.3201/eid2209.160140.
31. Babines-Orozco L, Balbuena-Alonso MG, Barrios-Villa E, Lozano-Zarain P, Martínez-Laguna Y, Rocha-Gracia RDC, Cortés-Cortés G. Antimicrobial resistance in food-associated *Escherichia coli* in Mexico and Latin America. Biosci Microbiota Food Health. 2024; 43(1): 4-12. DOI: 10.12938/bmfh.2023-022.
32. Dawadi P, Bista S, Bista S. Prevalence of colistin-resistant *Escherichia coli* from poultry in South Asian Developing Countries. Vet Med Int. 2021; 2021: 6398838. DOI: 10.1155/2021/6398838.

33. Barlaam A, Parisi A, Spinelli E, Caruso M, Taranto PD, Normanno G. Global emergence of colistin-resistant *Escherichia coli* in food chains and associated food safety implications: a review. J Food Protect. 2019; 82(8): 1440-1448.
DOI: 10.4315/0362-028X.JFP-19-116.

34. Nakayama T, Jinnai M, Kawahara R, Diep KT, Thang NN, Hoa TT, Hanh LK, Khai PN, Sumimura Y, Yamamoto Y. Frequent use of colistin-based drug treatment to eliminate extended-spectrum beta-lactamase-producing *Escherichia coli* in backyard chicken farms in Thai Binh Province, Vietnam. Trop Anim Health Prod. 2017; 49(1): 31-37.
DOI 10.1007/s11250-016-1154-y.

35. Saidani M, Messadi L, Sahmin E, Zouaoui S, Soudani A, Daaloul-Jedidi M, Mamlouk A, Chehida FB, Madec J-Y, Haenni M, Haenni M. ESBL-and mcr-1-producing *Escherichia coli* in veal calves in Tunisia. J Glob Antimicrob Resist. 2019; 104-105.
DOI: ff10.1016/j.jgar.2019.08.009f.

36. Haenni M, Beyrouthy R, Lupo A, Châtre P, Madec JY, Bonnet R. Epidemic spread of *Escherichia coli* ST744 isolates carrying mcr-3 and bla CTX-M-55 in cattle in France. J Antimicrob Chemother. 2018; 73(2): 533-536.
DOI: 10.1093/jac/dkx418.

37. El Garch F, Sauget M, Hocquet D, LeChaudee D, Woehrle F, Bertrand X. mcr-1 is borne by highly diverse *Escherichia coli* isolates since 2004 in food-producing animals in Europe. Clin Microbiol Infect. 2017; 23(1): 51.e1-51.e4.
DOI: 10.1016/j.cmi.2016.08.033.

38. Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, Alpuche-Aranda CM. Antimicrobial resistance: One Health approach. Vet World. 2022; 15(3): 743-749. DOI: 10.14202/vetworld.2022.743-749.

Table 1. Resistant profiles of *E. coli* isolates between 2020-2022

Year	Number of bacteria	ANTIBIOTICS										
		ENR	GEN	AMC	SXT	OT	CFP	N	CEQ	CFXK	TE	CT
2020	1	R	I	S	R	R	I	R	I	R	R	I
	1	R	R	R	R	R	R	R	R	R	R	I
	1	R	R	S	R	R	S	S	R	S	R	I
	1	R	R	I	R	R	R	R	S	R	R	I
	1	R	R	R	R	R	R	R	R	R	R	I

	1	S	R	R	R	R	R	R	R	R	R	I
	1	R	R	R	R	R	R	R	R	R	R	I
2021	1	R	I	R	R	R	R	R	R	R	R	R
	17*	R	R	R	R	R	R	R	R	R	R	R
	1	R	I	R	R	R	R	R	R	R	R	I
	2	R	I	R	R	R	R	R	R	R	R	R
	1	R	I	R	R	R	R	R	R	R	R	I
2022	24*	R	R	R	R	R	R	R	R	R	R	R
	1	S	R	R	I	R	R	R	R	R	R	R
TOTAL	54											

*, Haemolytic *E. coli*; ENR, enrofloxacin; GEN, gentamicin; AMC, amoxillin/cluvalanic acid; SXT, trimethoprim-sulfamethoxazole; OT; oxytetracycline; CFP, cephaperazone; N, neomycin; CEQ, cefquinom; CFXK, cephalexin/kanamycin, TE, tetracyclin; CT, colistin; R, resistance; I, Intermediate; S, Sensitive.

Table 2. MDR profiles of *E. coli* isolates

Number of *E. coli*	MDR profiles
41	ENR/GEN/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE/CT
1	GEN/AMC/OT/CFP/N/ CEQ/CFXK/TE/CT
1	ENR/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
2	ENR/ AMC/SXT/OT/CFP/N/CEQ/CFXK/TE/CT
1	ENR/ AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
1	ENR/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE/CT
1	ENR/GEN/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
1	GEN/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
1	ENR/GEN/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
1	ENR/GEN/SXT/OT/CFP/N/CFXK/TE
1	ENR/GEN/SXT/OT/CEQ/TE

1		ENR/GEN/AMC/SXT/OT/CFP/N/CEQ/CFXK/TE
1		ENR/SXT/OT/N/CFXK/TE

ENR, enrofloxacin; GEN, gentamicin; AMC, amoxillin/cluvalanic acid; SXT, trimethoprim-sulfamethoxazole; OT; oxytetracycline; CFP, cephaperazone; N, neomycin; CEQ, cefquinom; CFXK, cephalexin/kanamycin, TE, tetracyclin; CT, colistin.

Table 3. The increased beta-lactam and colistin resistance over the years

Year	**Number of *E. coli***	**Resistance profiles of beta-lactams and colistin**
	1	CFCK
2000	1	AMC/CFP/CEQ/CFXK
	1	CEQ
	1	CFP/CFXK
2001	5	AMC/CFP/CEQ/CFXK
	20	AMC/CFP/CEQ/CFXK/CT
2022	25	AMC/CFP/CEQ/CFXK/CT

AMC, amoxillin/cluvalanic acid; CFP, cephaperazone; CEQ, cefquinom; CFXK, cephalexin/kanamycin; CT, colistin.