

Phenotypic characterization of gentamicin-resistant *Escherichia coli* strains isolated from raw chicken meat samples in Türkiye: A public health concern

Caracterización fenotípica de cepas de *Escherichia coli* resistentes a la gentamicina aisladas de muestras de carne cruda de pollo en Turquía: Un problema de salud pública

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ABSTRACT

The inappropriate use of antibiotics in poultry production is considered to have contributed to the global increase and dissemination of multidrug-resistant pathogens. The objective of this study was to determine the occurrence of gentamicin-resistant *Escherichia coli* strains isolated from chicken meat samples and to evaluate their antimicrobial resistance profiles in Sivas province, Türkiye. A total of hundred fresh chicken meat samples (breast, drumstick, and wings) were collected from September to December 2022. After pre-enrichment of the samples material in buffered peptone water, the broths were streaked onto Tryptone Bile X-Glucuronide agar containing 8 µg/mL gentamicin. Antimicrobial susceptibility testing was performed using the broth microdilution method to determine minimum inhibitory concentrations. A total of 50 gentamicin-resistant *E. coli* strains were isolated from chicken meat samples. The strains showed high resistance to Ampicillin (100 %), followed by Trimethoprim/Sulfamethoxazole (88 %) and Ciprofloxacin (64 %), respectively. Multidrug-resistance was found in 96 % (n = 48) of the isolates. All gentamicin-resistant *E. coli* strains were susceptible to Amikacin, Carbapenems, and β-lactam/β-lactamase inhibitor combinations. The presence of resistant *E. coli* in raw chicken meat confirms its potential role of as source for the spread of antimicrobial resistance and multidrug-resistant strains in retail food. This study highlights the importance of continuous monitoring of *E. coli* strains isolated from raw chicken meat within the framework of the public health concept.

Key words: Antimicrobial resistance; chicken meat; *Escherichia coli*; gentamicin-resistant, multidrug-resistant strains; public health

RESUMEN

El uso inapropiado de antibióticos en la producción avícola se considera que ha contribuido al aumento y la diseminación global de patógenos resistentes a múltiples fármacos. El objetivo de este estudio fue determinar la presencia de *Escherichia coli* resistente a la gentamicina aislada de muestras de carne de pollo, centrándose en sus perfiles de resistencia a los antimicrobianos en la provincia de Sivas, Turquía. Se recolectaron y analizaron cien muestras de piezas de pollo fresco (pechuga, muslo y alas). Las muestras se recolectaron entre septiembre y diciembre de 2022. Después del enriquecimiento previo del material de muestra en agua de peptona tamponada, el caldo se sembró en agar Triptona Bilis X-glucurónico que contenía 8 µg/mL de gentamicina. Las pruebas de susceptibilidad a los antimicrobianos se realizaron utilizando el método de concentraciones inhibitorias mínimas por microdilución en caldo. Se aislaron un total de 50 cepas de *E. coli* resistente a la gentamicina de muestras de carne de pollo. Las cepas de *E. coli* mostraron una alta resistencia a la Ampicilina (100 %), seguida de Trimetoprima/sulfametoxazol (88 %) y Ciprofloxacina (64 %), respectivamente. Las cepas de *E. coli* resistente a la gentamicina presentaron una tasa de multiresistencia del 96 % (n = 48). Todas las cepas de *E. coli* resistente a la gentamicina fueron susceptibles a la Amikacina, los Carbapenémicos y las combinaciones de β-lactámicos/inhibidores de β-lactamasa. Se confirma que la *E. coli* presente en muestras de carne cruda de pollo es una fuente potencial de propagación de la resistencia a los antimicrobianos y de cepas multirresistentes. En este estudio se destaca la importancia del monitoreo continuo de cepas de *E. coli* aisladas de carne cruda de pollo en el marco del concepto de salud pública.

Palabras clave: Resistencia a los antimicrobianos; carne de pollo; *Escherichia coli*; resistente a la gentamicina; cepas multirresistentes; salud pública

INTRODUCTION

Antimicrobial resistance (AMR) continues to pose a global threat to public health, and resistance in zoonotic pathogens is of particular importance for food safety [1]. According to the World Health Organization (WHO) reports resistant bacteria belonging to the order Enterobacterales, such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, and *Salmonella* spp., are common pathogens in both humans and farm animals [1].

Antibiotics have been used in livestock farming since the 1950s for purposes such as growth promotion, treatment, and disease prevention. The rate of antimicrobial use is reported to be 73 % in intensive animal husbandry [2].

The use of antimicrobials as growth promoters in poultry feed has been prohibited in both the European Union (EU) and Türkiye since 2006 [3, 4]. These restrictions have significantly reduced antimicrobial usage in food-producing animals. Indeed, following the implementation of these regulations, antimicrobial use in the EU has decreased by 43.0 % [5]. Despite these measures, it is estimated that 99,502 tons of antimicrobials were used globally in 2020 and this number is expected to rise to 107,472 tons by 2030, with Asian countries accounting for the majority of users [6].

E. coli, a member of the order Enterobacterales, is a natural inhabitant of the intestinal microbiota in humans and animals. Nevertheless, pathogenic strains can cause various infections [7]. Food-borne AMR *E. coli* isolates are particularly concerning due to their ability to disseminate resistance genes via horizontal gene transfer, thereby posing significant risks to public health [8]. Numerous studies have shown that *E. coli* strains isolated from poultry, production environment, and derived products exhibit high resistance to multiple classes of antibiotics, reflecting a critical global health issue [9, 10, 11, 12]. Consequently, the EU, through a directive issued in 2021, designated *E. coli*, as an indicator microorganism for monitoring antimicrobial resistance in the intestinal flora of farm animals [11, 12, 13].

Gentamicin (CN), an aminoglycoside (AG) antibiotic, is widely used in both human and veterinary medicine for the treatment of bacterial infections owing to its broad-spectrum activity. Notably, aminoglycosides are included in the WHO list of critical important antibiotics, highlighting the need for prudent use to preserve their effectiveness [14, 15, 16, 17]. However, its therapeutic use in poultry production has led to the emergence and dissemination of gentamicin-resistant (CN^R) *E. coli* strains [16]. Previous studies have revealed that CN resistance can spread from poultry farms to humans through the food chain [16, 18]. In Canada, CN resistance was detected in *E. coli* isolates of both human and poultry origin [16]. Researchers found that the prevalence of CN^R *E. coli* isolates from poultry sources (e.g., clinical samples, feces/manure, and cecal contents) was 18.3 % between 2009-2013, and 18.4 % between 2014-2017, while the prevalence among *E. coli* isolates from retail chicken meat increased from 14.0 % to 21.4 %, suggesting a significant upward trend over time [16]. This data indicates that the prevalence of CN^R *E. coli* isolates from chicken meat sold for sale is increasing. In addition to direct CN exposure, resistance to CN may also be maintained through co-selection driven by the use of other antimicrobial classes in poultry production. Genomic investigations within a One Health framework have demonstrated that CN resistance genes are frequently co-located with resistance determinants to β -lactams, sulfonamides, and fluoroquinolones (FQ) on mobile genetic elements shared

between human and poultry associated *E. coli* populations. This suggests that the use of non-aminoglycoside antimicrobials at the farm level may indirectly select for CN^R strains throughout the agri-food chain [16].

In Türkiye, although the AMR profiles of *E. coli* strains isolated from poultry, poultry production environments, and poultry products have been investigated, there is limited information regarding CN resistance patterns and underlying mechanisms [9, 19, 20]. A recent study Şahin *et al.* [12], reported a CN rate of 15.8 % among *E. coli* strains isolated from poultry cecal samples collected from different poultry companies [12].

One of the primary mechanisms involved in CN^R is the presence of genes encoding aminoglycoside-modifying enzymes (AMEs), such as *aac(3)-IIa*, *aac(6')-Ib*, and *ant(2'')-Ia*, which reduce the efficacy of the antibiotic [21]. The horizontal transmission of these genes through mobile genetic elements represents a global public health concern [14].

In particular, contamination from chicken meat serves as a significant vehicle for the transmission of resistant strains to humans, emphasizing the importance of this issue within the framework of food safety and the One Health approach [22]. Therefore, the present study aimed to determine the antimicrobial resistance profiles of phenotypically CN^R *E. coli* strains isolated from chicken meat in Türkiye. The susceptibility of the isolates to various antibiotics was assessed based on minimum inhibitory concentration (MIC) values. The study contributes to the phenotypic characterization of CN^R *E. coli* strains from chicken meat samples and provides valuable insights into the potential public health risks associated with these resistance patterns.

MATERIALS AND METHODS

Chicken meat sample collection and isolation of gentamicin-resistant *E. coli*

A total of 100 raw chicken meat samples (breast, drumstick, and wings) were collected from various supermarkets, local markets and butchers in Sivas region of Türkiye from September to December 2022. For the isolation of *E. coli*, 10 g of sample was aseptically each chicken meat (breast, drumstick and wing) using sterile instruments. The sample was transferred into 90 mL of sterile Buffered Peptone Water (BPW; Oxoid CM0509, United Kingdom) and homogenized for 1-2 min using a stomacher (Interscience Bag Mixer 400, France). The homogenates were incubated at 37 ± 1 °C for 24 h in incubator (Binder BD115, Germany) [23]. Following incubation, 100 µL of the enrichment culture was plated onto Tryptone Bile X-Glucuronide agar (TBX; Oxoid CM0945, United Kingdom) supplemented with 8 µg/mL CN (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 ± 1 °C for 24 h. After incubation typical green colonies presumed to be *E. coli* (one colony per sample) were sub-cultured on Columbia Agar supplemented with 5 % sheep blood (CBA; Oxoid CM0331, United Kingdom) and incubated at 37 ± 1 °C for 24 h [24].

Pure colonies were preserved in Tryptone Soya Broth (TSB; Oxoid CM0129, United Kingdom) containing 20 % glycerol and stored at -20 °C in deep freezer (Bosch GSN33VWEON, Poland). Identification of *E. coli* isolates was performed using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonics GmbH &

Co. KG, Bremen, Germany). For MALDI-TOF MS identification, a fresh bacterial colony grown on CBA was transferred onto a polished steel target plate and overlaid with 1 µL of α-cyano-4-hydroxycinnamic acid matrix solution. After air-drying, spectra were acquired using the Bruker Biotyper system according to the manufacturer's instructions, and identification was performed by comparison with the reference database. Only isolates with MALDI-TOF MS scores ≥ 2.3 were accepted as *E. coli* at the species level [25]. Preliminary confirmation of CN^R *E. coli* strains was carried out using the disk diffusion method, and definitive phenotypic resistance assessment was performed by the broth microdilution technique to determine MIC values, in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [26].

Antimicrobial susceptibility testing using broth microdilution

The *in vitro* antimicrobial susceptibility of the CN^R *E. coli* strains was determined using broth microdilution with the BD Phoenix™ automated test panels (NMIC-433, Becton Dickinson, Sparks, MD, USA). All susceptibility tests were performed according to the manufacturer's instructions. Each *E. coli* strains was tested against the following antimicrobial agents: Amikacin (AK), CN, Ampicillin (AMP), Amoxicillin/Clavulanate (AMC), Ampicillin/Sulbactam (A/S), Piperacillin/Tazobactam (P/T), Cefazolin (CFZ), Cefuroxime (CXM), Ceftriaxone (AXO), Ceftazidime (CAZ), Cefepime (FEP), Ceftolozane/Tazobactam (C/T), Ciprofloxacin (CIP), Levofloxacin (LEV), Ertapenem (ETP), Imipenem (IMI), Meropenem (MERO), Colistin (CT), Tigecycline (TGC) and Trimethoprim/Sulfamethoxazole (SXT). The CT, TGC, C/T, P/T, ETP, IMI, and MERO are authorized for use in human medicine. These antimicrobials were included in the study because they are part of the standardized NMIC surveillance panel.

Antimicrobial susceptibility testing was performed in accordance with the recommendations of the EUCAST [26]. MIC values were interpreted using EUCAST epidemiological cut-off values (ECOFF). Isolates with MIC values at or below the ECOFF were classified as wild-type (WT), indicating the absence of acquired resistance mechanisms, whereas isolates with MIC values above the ECOFF were classified as non-wild-type (NWT). For descriptive purposes, WT isolates were considered phenotypically susceptible, while NWT isolates were considered resistant. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. The *E. coli* ATCC 25922 was used as a quality control strain, as recommended by EUCAST.

RESULTS AND DISCUSSION

Antimicrobial susceptibility testing of CN^R *E. coli* strains (n = 50) obtained from chicken meat samples revealed varying levels of resistance to across different classes of antibiotics. The highest resistance rates were found for 100 % to AMP and 88 % to SXT. High levels of resistance were also detected against 64 % to CIP, 62 % to LEV, 50 % to AMC and CFZ, 48 % to AXO and CXM, and 42 % to A/S, respectively. The lowest resistance rates were 2 % to CT and TGC (TABLE I; FIG. 1). On the other hand, all *E. coli* strains were found to be susceptible to AK, C/T, P/T, ETP, IMI, and MERO. In the present study, 96 % (n = 48) of the strains exhibited resistance to at least three different classes of antibiotics and were therefore classified as MDR. These results demonstrate that multiple antibiotic resistance is widespread among chicken derived CN^R *E. coli* strains. The high resistance rates against β-lactam, folate pathway inhibitor, and FQ antibiotics, in addition to CN resistance, highlight the potential public health risks associated with the consumption of contaminated chicken meat (TABLE I).

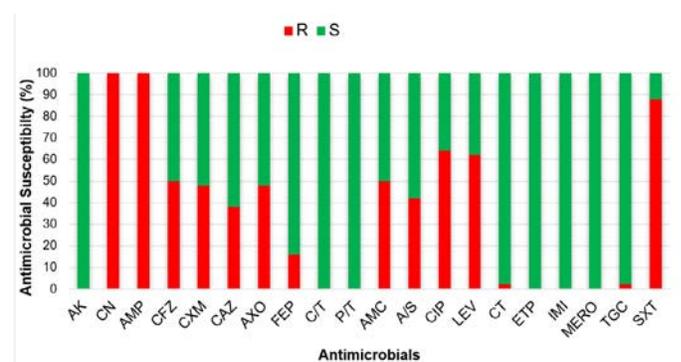


FIGURE 1. MIC based antimicrobial susceptibility profiles of CN^R *E. coli* strains from chicken meat. Amikacin (AK), Gentamicin (CN), Ampicillin (AMP), Amoxicillin/Clavulanate (AMC), Ampicillin/Sulbactam (A/S), Piperacillin/Tazobactam (P/T), Cefazolin (CFZ), Cefuroxime (CXM), Ceftriaxone (AXO), Ceftazidime (CAZ), Cefepime (FEP), Ceftolozane/Tazobactam (C/T), Ciprofloxacin (CIP), Levofloxacin (LEV), Ertapenem (ETP), Imipenem (IMI), Meropenem (MERO), Colistin (CT), Tigecycline (TGC) and Trimethoprim/Sulfamethoxazole (SXT) R: Resistant S: Susceptible

TABLE I
MIC distribution of gentamicin-resistant *Escherichia coli* strains from chicken meat samples (n; %)

Antibiotic Class	Antimicrobial Agent	ECOFF Value (µg/mL)	MIC Range (µg/mL)	R	S
				n (%)	
Aminoglycoside	Amikacin (AK)	> 8	8-32	0	50 (100 %)
	Gentamicin (CN)	> 2	2-8	50 (100 %)	0
β-lactam Penicillins	Ampicillin (AMP)	> 8	4-16	50 (100 %)	0
1 st Generation Cephalosporins	Cefazolin (CFZ)	> 4	4-32	25 (50 %)	25 (50 %)
2 nd Generation Cephalosporins	Cefuroxime (CXM)	> 8	4-16	24 (48 %)	26 (52 %)
3 rd Generation Cephalosporins	Ceftazidime (CAZ)	> 4	1-8	19 (38 %)	31 (62 %)
	Ceftriaxone (AXO)	> 2	1-4	24 (48 %)	26 (52 %)
4 th Generation Cephalosporins	Cefepime (FEP)	> 4	1-8	8 (16 %)	42 (84 %)
3 rd Generation Cephalosporins with β-lactamase inhibitors	Ceftolozane/Tazobactam (C/T)	> 2	2/4-8/4	0	50 (100 %)
Aminopenicillins with β- lactamase inhibitors	Piperacillin/Tazobactam (P/T)	> 8	4/4-16/4	0	50 (100 %)
	Amoxicillin/Clavulanate (AMC)	> 8	2/2-16/2	25 (50 %)	25 (50 %)
	Ampicillin/Sulbactam (A/S)	> 8	1/8- 8/8	21 (42 %)	29 (58 %)
Fluoroquinolones	Ciprofloxacin (CIP)	> 0.5	0.0625-1	32 (64 %)	18 (36 %)
	Levofloxacin (LEV)	> 1	0.5-2	31 (62 %)	19 (38 %)
Polymyxins	Colistin (CT)	> 2	1-4	1 (2 %)	49 (98 %)
Carbapenems	Ertapenem (ETP)	> 0.5	0.25-1	0	50 (100 %)
	Imipenem (IMI)	> 4	0.25-8	0	50 (100 %)
	Meropenem (MERO)	> 8	0.125-8	0	50 (100 %)
Glycylcycline	Tigecycline (TGC)	> 0.5	0.5-2	1 (2 %)	49 (98 %)
Folate pathway inhibitors	Trimethoprim/ Sulfamethoxazole (SXT)	> 4	2/38- 8/152	44 (88 %)	6 (12 %)

n = 50 strains. R: Resistance S: Susceptible n: number of isolates; %: percentage of isolates. Breakpoints were applied according to EUCAST. ECOFF: Epidemiological cut-off values

Gentamicin a member of the aminoglycoside antibiotic class, is an important antimicrobial agent widely used in both Veterinary Medicine and human healthcare. It has long been applied in poultry production, particularly for the treatment of septicemia, colibacillosis, and respiratory tract infections caused by *E. coli* [27]. However, because of its requirement for parenteral administration and the potential risk of tissue residues, the use of CN in the poultry sector has been reportedly declined in recent years [28].

Despite this, the high rate of resistant strains still being isolated indicates the extensive historical use of CN and the genetic transferability of aminoglycoside resistance. CN resistance is most commonly mediated by AMEs, including aminoglycoside acetyltransferases, O-nucleotidyltransferases, and O-phosphotransferases, which enzymatically inactivate the drug [16]. CN resistance is often mediated by plasmid-borne resistance genes. These plasmids may co-harbor genes conferring resistance not only to aminoglycosides but also to β-lactam, folate pathway inhibitor, and FQ antibiotics [29].

The localization of these resistance genes on mobile genetic elements facilitates their horizontal transfer and long-term maintenance in the agri-food chain, even in the absence of current selective pressure [30]. Although the use of CN

in Türkiye is currently subject to strict regulatory control in line with EU regulations, relatively high resistance rates are still reported. Indeed, CN resistance among *E. coli* isolates from different poultry farms have been reported to range between 9.8 and 22.9 % [12]. This observation may reflect the impact of historical antimicrobial use and the persistence of aminoglycoside resistance genes within poultry-associated bacterial populations.

Due to the absence of maximum residue limits for CN in poultry within the EU, *in ovo* administration or any other application of CN in poultry production is not permitted [31]. Consequently, low levels of CN resistance are generally reported among poultry-associated *E. coli* isolates in the EU, reflecting the effectiveness of regulatory restrictions and antimicrobial monitoring policies.

Furthermore, the level of CN resistance in indicator *E. coli* isolates from broilers and fattening turkeys across EU Member States has been reported to be consistently low, ranging between 2.8 % and 3.5 % [32]. In contrast, higher frequencies of CN resistance have been reported in conventionally raised poultry in countries where *in ovo* CN use has historically been practiced, such as the United States [33]. Davies *et al.* [33] demonstrated that CN resistance was significantly more prevalent among *E.*

Gentamicin-resistant *E. coli* in chicken meat samples / Seyda Şahin

coli isolates from conventionally raised broilers and suggested that the use of CN supplemented *in ovo* vaccines may act as an important selective factor for resistance development in poultry production systems [18, 33, 34].

The present study analyzed retail chicken meat rather than on-farm samples, CN^R *E. coli* detected at the retail level may originate from colonized birds carrying resistant strains from the production phase, which can persist through slaughter and processing. In this context, the use of CN supplemented *in ovo* vaccines has been suggested as a potential factor for the initial selection of resistant *E. coli* in broilers, which may subsequently contaminate carcasses and meat products along the food chain.

In addition, cross-contamination during slaughtering, processing, and retail handling may further contribute to the presence of resistant bacteria in chicken meat. Together, these observations highlight that CN resistance detected in poultry meat likely reflects cumulative effects of antimicrobial use practices and food chain dynamics rather than a single point source.

The nonexistent resistance to AK found in this study is in line with what is known about the structure of this aminoglycoside. AK is a semisynthetic aminoglycoside that was made to avoid most AMEs. These enzymes are the main way that this class of antibiotics can become resistant. AK, in contrast to other aminoglycosides, is predominantly resistant to enzymatic inactivation by acetyltransferases, phosphotransferases, and nucleotidyl transferases. Resistance to AK is primarily linked to acetylation facilitated by aminoglycoside 6-N-acetyltransferase type Ib, an enzyme encoded by genes commonly found on mobile genetic elements like plasmids, integrons, and transposons in Gram-negative bacteria. The lack of phenotypic resistance to AK in this isolates may indicate the limited prevalence of such resistance determinants in poultry associated *E. coli*, alongside the constrained application of amikacin in food-producing animals [35, 36].

In addition to CN^R *E. coli*, the present study was found a similarly high rate of phenotypic resistance to β -lactam, folate pathway inhibitor, and FQ antibiotics (TABLE I). Therefore, the detection of CN^R *E. coli* in retail poultry meat suggests a potential role of poultry products as reservoirs for MDR bacteria, although contamination may occur at various points along the food chain, from production to retail handling. However, one of the limitations of this study is that resistance was evaluated only at the phenotypic level, and aminoglycoside resistance genes were not examined genotypically.

The findings of this study are generally consistent with reports from several studies. Although direct comparison of AMR prevalence between broiler isolates derived from (e.g., cecal or farm samples) and retail chicken meat is limited by differences in sampling matrices, several studies, including our previous findings on poultry cecal isolates in Türkiye, indicate that AMR *E. coli* is already established at the production level [12, 37, 38].

The detection of resistant *E. coli* in retail poultry meat reported in multiple studies further suggests that these resistant populations may persist and be disseminated along the agri-food chain, from primary production to market products. For instance, in a recent comparative analysis of conventional and antibiotic-free retail chicken meat, *E. coli* was detected in

approximately 20.3 % of samples, and MDR resistance (38.2 %), including resistance to AMP, SXT, and TET was prevalent across both production systems, highlighting persistent AMR in poultry meat products [39]. Similarly, in a study from Romania analyzing chicken meat, *E. coli* isolates frequently exhibited high resistance rates to multiple antibiotic classes including TET (80 %), AMP (80 %), SMX (73,33 %), CHL (70 %), nalidixic acid (NAL) (60 %), CIP (56.66 %), CTX (46.66 %), CAZ (43.33 %) and CN (40 %), underscoring the public health relevance of AMR in poultry meat. Moreover, more than 70 % of the isolates proved to be MDR [40]. Consistent with previous work retail poultry products, these findings emphasize that AMR in poultry meat reflects a complex interplay of production practices, bacterial contamination, and food chain dynamics rather than a single point source [41].

It is estimated that in most developed countries, approximately 50-80 % of all antibiotics produced are administered to food-producing animals [5]. Antimicrobials such as AMP, SXT, and TET have long been used in veterinary medicine to prevention and treatment in food animals [12].

According to the WHO classification, AMP, SMX, and TET are categorized as “highly important antimicrobials” due to their essential role in human medicine [17]. According to the latest report from the EU, *E. coli* strains have shown the highest resistance rates to AMP, SXT and TET [30]. In the present study, CN^R *E. coli* strains exhibited 100 % resistance to AMP, 88 % to SXT, 64 % to CIP, and 62 % to LEV.

These findings suggest that CN^R *E. coli* strains may serve as an important reservoir for antimicrobial resistance genes across different antibiotic classes. Furthermore, resistance to CIP and NAL has also been reported as widespread among poultry isolates in European countries [32], which could exert selection pressure explaining the high percentage of resistance observed in the present study.

The findings of this study regarding the resistance profiles of *E. coli* strains are largely consistent with the data reported by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) [36]. These results confirm the high levels of resistance of *E. coli* strains to AMP, SXT, and CIP as well as the limited resistance to CT, TGC. All CN^R *E. coli* strains showed 100 % susceptibility to P/T, C/T, and carbapenems (ETP, IMI, and MERO).

This is a positive finding, as these antimicrobials are primarily reserved for human medicine and are not authorized for use in livestock production. The limited resistance found likely reflects reduced selective pressure in the poultry sector, highlighting the effectiveness of antimicrobial stewardship and regulatory policies [36]. However, sporadic detection of resistance to last resort antimicrobials, including the identification of TGC resistant and *mcr-1* *E. coli* in poultry and poultry meat samples in Türkiye, underscores the need for continuous and integrated antimicrobial resistance surveillance along the agri-food chain [1, 36, 40, 42, 43].

Fluoroquinolones are commonly used as first-line antibiotics in the treatment of *E. coli* infections [44]. These agents are widely prescribed to manage bacterial infections in humans, poultry and other animals. Enrofloxacin (ENR), a member of the FQ class is frequently administered by veterinarians to prevent early chick mortality and reduce disease transmission [45].

However, *E. coli* strains obtained from poultry meat in Türkiye have been reported to exhibit high levels of resistance, reaching up to 100 %, to quinolones/FQ including CIP, ENR, LEV, Nalidixic acid (NAL), and Norfloxacin [9].

These findings should serve as a warning to the poultry industry to exercise caution in the use of FQ during production. In this context, further studies with larger sample sizes are needed to better determine the prevalence and mechanisms of FQ resistance. In the present study, resistance to FQ antibiotics was showed at 64 % for CIP and 62 % for LEV (TABLE I). EFSA/ECDC [36] reported similarly high resistance rates, with 54.3 % CIP resistance detected in in *E. coli* isolates from broilers. Similarly, high levels of resistance to FQ have also been reported by Bratfelan *et al.* [40] in *E. coli* isolates obtained from chicken meat samples in Romania, with a CIP resistance rate of 56.66 %. In addition, Habib *et al.* [46] found that 80 % of supermarket chicken meat derived extended-spectrum β -lactamase (ESBL)-producing *E. coli* isolates in the United Arab Emirates were resistant to CIP. These findings support the global nature of FQ resistance among *E. coli* in poultry products and align with the high CIP resistance observed in the present study.

Trimethoprim/Sulfamethoxazole shows bacteriostatic properties when used alone; however, its combination produces a bactericidal effect by successively inhibiting two enzymes in the folate synthesis pathway [47]. In the present study, resistance to SXT was detected in 88 % CN^R *E. coli* strains. Similarly, several studies have been reported high levels of SXT resistance in *E. coli* isolates from chicken meat samples in Brazil and Romania [39, 40].

According to recent reports, Aminopenicillin resistance is common among commensal *E. coli* isolates from food-producing animals in EU countries. The prevalence was reported as 44.7 % in broilers and 55.2 % in turkeys [48]. AMC, which belongs to the aminopenicillin class, has been commercially available since 1981 and is widely used in European countries to treat a variety of infections in both humans and animals. However, this extensive use may contribute to the development of acquired resistance in both pathogenic and commensal bacteria [49].

The findings from this study revealed that resistance to AMC was 50 % among CN^R *E. coli* strains. In addition, resistance to third-generation cephalosporins was found to be 38-48 % of the isolates for CAZ and AXO, respectively. It has been reported that AMC resistance may facilitate the selection and dissemination of ESBL and AmpC β -lactamase producing bacteria due to its overlapping activity spectrum with second and partially third-generation cephalosporins [12, 49]. These results are consistent with the findings of the present study. However, there remains a lack of comprehensive data on how the AMC combination specifically selects for resistance in animals.

CONCLUSION

This study demonstrated that raw chicken meat available for consumers in Türkiye was frequently contaminated with CN^R *E. coli* strains. The research provides comprehensive phenotypic data on CN^R *E. coli* strains collected from chicken meat samples in Sivas province, Türkiye. A high prevalence of resistance to AMP, SXT and CIP was found, indicating resistance to multiple classes of antimicrobials among the strains. These findings suggest that the consumption of undercooked chicken meat or foods cross-contaminated with CN^R *E. coli* strains may pose a significant risk to public health and highlight the need

for continuous monitoring and responsible antimicrobial use in poultry production.

In this context, more research utilizing MIC based techniques would provide a better assessment of resistance phenotypes and improve surveillance of AMR in the poultry food chain. Taken together, these findings highlight that AMR in the poultry food chain is not confined to a single production stage or country. Accordingly, effective control strategies should rely on coordinated, policy based actions supported by governmental regulations. In parallel, preventive measures such as improved biosecurity, vaccination, and better hygiene are essential to reduce the reliance on antimicrobials.

Conflict of interest

The author declare no potential conflicts of interest.

Ethics approval

Ethical approval is not required for this study.

BIBLIOGRAPHICS REFERENCES

- [1] World Health Organization (WHO). Report signals increasing resistance to antibiotics in bacterial infections in humans and need for better data. [Internet]. Geneva, Suiza: World Health Organization; 2022. [Accessed 14 Jan 2025]. Available in: <https://goo.su/w3RPDGy>
- [2] Morrow CJ. Antimicrobial resistance (AMR): An important one health issue for layer and meat poultry industries worldwide. *Poult. Sci.* [Internet]. 2024; 103(7):103690. doi: <https://doi.org/qcwg>
- [3] Official Newspaper of the Republic of Türkiye. Yem Katkıları ve Premikslerin Üretimi, İthalatı, İhracatı, Satışı ve Kullanımı Hakkında Tebliğde Değişiklik Yapılmasına Dair Tebliğ. [Internet]. Tarım ve Köyişleri Bakanlığında. 21 Ocak 2006. Sayı: 26056, Tebliğ No: 2006/1 Resmi Gazete, Ankara. [Accessed 8 Nov 2025]. Available in: <https://goo.su/3PdTVU>
- [4] Phillips I. Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. *Int. J. Antimicrob. Agents.* [Internet]. 2007; 30(2):101-107. doi: <https://doi.org/dgd3tg>
- [5] European Medicines Agency (EMA), European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 31 European countries in 2022. [Internet]. (EMA/299538/2023). Luxembourg: Publications Office of the European Union. 2023 [Accessed 8 Nov 2025]. 94 p. Available in: <https://goo.su/rBOrc>
- [6] Mulchandani R, Wang Y, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. *PLOS Glob. Public Health.* [Internet]. 2023; 3(2):e0001305. doi: <https://doi.org/g6d6g>
- [7] van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics: Links between animals and humans. *Int. J. Antimicrob. Agents.* [Internet]. 2000; 14(4):327-335. doi: <https://doi.org/c44dzq>

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- [8] Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.* [Internet]. 2011; 24(4):718-733. doi: <https://doi.org/c2s5j4>
- [9] Şahin S. Determination of the ciprofloxacin-resistant *Escherichia coli* isolated from chicken meat in Turkey. *J. Hellenic Vet. Med. Soc.* [Internet]. 2020; 71(3):2291-2300. doi: <https://doi.org/qrg8>
- [10] Islam MS, Hossain MJ, Sobur MA, Punom SA, Rahman AMM, Rahman MT. A Systematic Review on the occurrence of antimicrobial-resistant *Escherichia coli* in poultry and poultry environments in Bangladesh between 2010 and 2021. *BioMed Res. Int.* [Internet]. 2023; 2023:2425564. doi: <https://doi.org/qrg9>
- [11] De Koster S, Ringenier M, Xavier BB, Lammens C, De Coninck D, De Bruyne K, Mensaert K, Kluytmans-van den Bergh M, Kluytmans J, Dewulf J, Goossens H, i-4-1-Health Study Group. Genetic characterization of ESBL-producing and ciprofloxacin-resistant *Escherichia coli* from Belgian broilers and pigs. *Front. Microbiol.* [Internet]. 2023; 14:1150470. doi: <https://doi.org/qrhb>
- [12] Kasanga M, Shempela DM, Daka V, Mwikisa MJ, Sikalima J, Chanda D, Mudenda S. Antimicrobial resistance profiles of *Escherichia coli* isolated from clinical and environmental samples: findings and implications. *JAC-Antimicrobial Resistance.* [Internet]. 2024; 6(2):dlae061. doi: <https://doi.org/qrtt>
- [13] European Commission (EC). Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal Bacteria and Repealing Implementing Decision 2013/652/EU. [Internet]. Official Journal of the European Union, 50:8-21, 2020 [Accessed 14 Jan 2024]. Available in: <https://goo.su/V7bUz>
- [14] Ruppé É, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Ann. Intensive Care.* [Internet]. 2015; 5(1):21. doi: <https://doi.org/f75pni>
- [15] Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. *Cold Spring Harb. Perspect. Med.* [Internet]. 2016; 6(6):a027029. doi: <https://doi.org/ghv48z>
- [16] Cox GW, Avery BP, Parmley EJ, Irwin RJ, Reid-Smith RJ, Deckert AE, Finley RL, Daignault D, Zhanel GG, Mulvey MR, Bharat A. A one health genomic investigation of gentamicin resistance in *Escherichia coli* from human and chicken sources in Canada, 2014 to 2017. *Antimicrob. Agents Chemother.* [Internet]. 2022; 66(10):e00677-22. doi: <https://doi.org/qrhc>
- [17] World Health Organization (WHO). WHO's list of medically important antimicrobials: a risk management tool for mitigating antimicrobial resistance due to non-human use. [Internet]. Geneva (CH): World Health Organization. 2024 [Accessed 14 Jan 2025]. 41 p. Available in: <https://goo.su/Uphbh3f>
- [18] Roedel A, Vincze S, Projahn M, Roesler U, Robé C, Hammerl JA, Noll M, Dahouk SA, Dieckmann R. Genetic but no phenotypic associations between biocide tolerance and antibiotic resistance in *Escherichia coli* from German broiler fattening farms. *Microorganisms.* [Internet]. 2021; 9(3):651. doi: <https://doi.org/qrhd>
- [19] Aslantaş Ö. Antimicrobial resistance among commensal *Escherichia coli* from broilers in Turkey. *Isr. J. Vet. Med.* [Internet]. 2018 [Accessed 14 June 2025].; 73(3):19-24. Available in: <https://goo.su/UrUul>
- [20] Şahin S, Mogulkoc MN, Kürekcı C. Disinfectant and heavy metal resistance profiles in extended spectrum β -lactamase (ESBL) producing *Escherichia coli* isolates from chicken meat samples. *Int. J. Food Microbiol.* [Internet]. 2022; 377:109831. doi: <https://doi.org/qrhf>
- [21] Nayme K, Barguigua A, Diawara I, El Otmani F, Elmdaghri N, Zerouali K, Timinouni M. Occurrence of genes encoding aminoglycoside-modifying enzymes in *Escherichia coli* isolates from chicken meat. *Br. Poult. Sci.* [Internet]. 2019; 60(6):798-801. doi: <https://doi.org/qrhg>
- [22] Hossain MMK, Islam MS, Uddin MS, Rahman AM, Ud-Daula A, Islam MA, Rubaya R, Bhuiya AA, Alim MA, Jahan N, Li J, Alam J. Isolation, identification and genetic characterization of antibiotic resistant *Escherichia coli* from frozen chicken meat obtained from supermarkets at Dhaka City in Bangladesh. *Antibiotics.* [Internet]. 2023; 12(1):41. doi: <https://doi.org/m7nq>
- [23] International Organization for Standard (ISO) 6887-2. Microbiology of the food chain-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products. 2nd edition. Geneva Switzerland: ISO. [Internet]. 2017 [Accessed 14 Jun 2025]. Available in: <https://goo.su/XcPo>
- [24] International Organization for Standard (ISO) 16649-2. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. 1st edition. Geneva Switzerland: ISO. [Internet]. 2001 [Accessed 14 Jun 2025]. Available in: <https://goo.su/n9YV>
- [25] Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. *Clin. Microbiol. Rev.* [Internet]. 2013; 26(3):547-603. doi: <https://doi.org/f49z2d>
- [26] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0. valid from 2024-01-01. [Internet]. Sweden: EUCAST. 2024 [Accessed 16 Jul 2024]. Available in: <https://goo.su/v99aH>
- [27] Landoni MF, Albarellos G. The use of antimicrobial agents in broiler chickens. *Vet. J.* 2015; 205(1):21-27. doi: <https://doi.org/ggj5n3>
- [28] European Medicines Agency (EMA). European Surveillance of Veterinary Antimicrobial Consumption. *Sales of veterinary antimicrobial agents in 31 European countries in 2019 and 2020.* [Internet]. (EMA/58183/2021). Luxembourg: Publications Office of the European Union. 2021 [Accessed 8 Nov 2025]. 130 p. Available in: <https://goo.su/HccpGLX>
- [29] Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S. Antimicrobial resistance in *Escherichia coli*. *Microbiol. Spectr.* [Internet]. 2018; 6(4):ARBA-0026-2017. doi: <https://doi.org/gdv75z>

- [30] Samtiya M, Matthews KR, Dhewa T, Puniya AK. Antimicrobial resistance in the food chain: trends, mechanisms, pathways, and possible regulation strategies. *Foods*. [Internet]. 2022; 11(19):2966. doi: <https://doi.org/qrqk>
- [31] European Medicines Agency (EMA). *Sales of veterinary antimicrobial agents in 30 European countries in 2016: Trends from 2010 to 2016*. Eighth ESVAC Report. [Internet]. (EMA/275982/2018). Luxembourg: Publications Office of the European Union. 2018; [Accessed 16 Jul 2024]. 184 p. Available in: <https://goo.su/cofaXf1>
- [32] European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2020/2021. *EFSA J*. [Internet]. 2023; 21(3):e07867. doi: <https://doi.org/qrqs>
- [33] Davies AR, Chisnall T, Akter S, Afrad MMH, Sadekuzzaman M, Badhy SC, Hasan Z, Rahman T, Smith RP, Card RM, Brum E, Chowdhury MGA. Genomic characterisation of *Escherichia coli* isolated from poultry at retail through sink surveillance in Dhaka, Bangladesh reveals high levels of multi-drug resistance. *Front. Microbiol*. [Internet]. 2024; 15:1418476. doi: <https://doi.org/qrqt>
- [34] Koike S, Mackie R, Aminov R. Agricultural use of antibiotics and antibiotic resistance. In: Mirete S, Pérez ML, editors. *Antibiotic Resistance Genes in Natural Environments and Long-Term Effects*. Aberdeen, United Kingdom: Nova Science Publishers, Inc. 2017 [Accessed 15 Jun 2025]; p. 217-250. Available in: <https://goo.su/1luOEU>
- [35] Ramirez MS, Tolmasky ME. Amikacin: uses, resistance, and prospects for inhibition. *Molecules*. [Internet]. 2017; 22(12):2267. doi: <https://doi.org/gct29m>
- [36] European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). Plain Language Summary of the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2022-2023. *EFSA J*. [Internet]. 2025 [Accessed 14 Jan 2025]; 23(3):ep230301. doi: <https://doi.org/qrqx>
- [37] Musa L, Stefanetti V, Casagrande-Proietti P, Grilli G, Gobbi M, Toppi V, Brustenga L, Magistrali CF, Franciosini MP. Antimicrobial Susceptibility of Commensal *E. coli* Isolated from Wild Birds in Umbria (Central Italy). *Animals*. [Internet]. 2023; 13(11):1776. doi: <https://doi.org/qrq5>
- [38] Grobbel M, Hammerl JA, Alt K, Irrgang A, Kaesbohrer A, Tenhagen BA. Comparison of antimicrobial resistances in *Escherichia coli* from conventionally and organic farmed poultry from Germany. *Antibiotics*. [Internet]. 2022; 11(10):1282. doi: <https://doi.org/qrq6>
- [39] Cerqueira-Cézar CK, Sampaio ANDCE, Caron EFF, Dellaqua TT, Ribeiro LFM, Tadielo LE, de Figueiredo-Pantoja JC, Fernandes-Viana GG, Marques-Rossi GA, Spanu C, Possebon FS, Pereira JG. Antimicrobial resistance in chicken meat: Comparing *Salmonella*, *Escherichia coli*, and *Enterococcus* from conventional and antibiotic-free productions. *Microorganisms*. [Internet]. 2025; 13(10):2227. doi: <https://doi.org/qrq8>
- [40] Brätfelan DO, Tabaran A, Colobatiu L, Mihaiu R, Mihaiu M. Prevalence and antimicrobial resistance of *Escherichia coli* isolates from chicken meat in Romania. *Animals*. [Internet]. 2023; 13(22):3488. doi: <https://doi.org/qrrc>
- [41] Davis GS, Waits K, Nordstrom L, Grande H, Weaver B, Papp K, Horwinski J, Koch B, Hungate BA, Liu CM, Price LB. Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC Microbiol*. [Internet]. 2018; 18(1):174. doi: <https://doi.org/gnnrp2>
- [42] Kurekci C, Aydin M, Nalbantoglu OU, Gundogdu A. First report of *Escherichia coli* carrying the mobile colistin resistance gene *mcr-1* in Turkey. *J. Glob. Antimicrob. Resist.* [Internet]. 2018; 15:169-170. doi: <https://doi.org/qrnn>
- [43] Kürekci C, Lu X, Sahin S, Celil BG, Soyer Y, Yeniköylü F, Wang Z, Li, R. Detection and Whole-Genome Analysis of tigeicycline resistant *Escherichia coli* in poultry and meat samples in Türkiye. *Poult. Sci.* [Internet]. 2025;104(2);104707. doi: <https://doi.org/qrnn>
- [44] Thompson D, Xu J, Ischia J, Bolton D. Fluoroquinolone resistance in urinary tract infections: Epidemiology, mechanisms of action and management strategies. *BJUI Compass*. [Internet]. 2024; 5(1):5-11. doi: <https://doi.org/hbknbv>
- [45] Moniri R, Dastehgoli K. Fluoroquinolone resistant *Escherichia coli* isolated from healthy broilers with previous exposure to fluoroquinolones: Is there a link? *Microb. Ecol. Health Dis.* [Internet]. 2005; 17(2):69-74. doi: <https://doi.org/cr5j49>
- [46] Habib I, Elbediwi M, Mohamed MYI, Ghazawi A, Abdalla A, Khalifa HO, Khan M. Enumeration, antimicrobial resistance and genomic characterization of extended-spectrum-lactamases producing *Escherichia coli* from supermarket chicken meat in the United Arab Emirates. *Int. J. Food Microbiol.* [Internet]. 2023; 398:110224. doi: <https://doi.org/qrqq>
- [47] Aktaş F. Sulfonamides, trimethoprim, and trimethoprim/sulfamethoxazole. *Turk. Klin. J. Infect. Dis. – Spec. Top.* 2017 [Accessed 15 Jun 2025]; 10(1):117-120. Available in: <https://goo.su/uH8MGH>
- [48] European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2019-2020. *EFSA J*. [Internet]. 2022; 20(3):e07209. doi: <https://doi.org/qrq3>
- [49] Van Duijkeren E, Rantala M, Bouchard D, Busani L, Catry B, Kaspar H, Pomba C, Moreno MA, Nilsson O, Ružauskas M, Sanders P, Teale C, Wester AL, Ignate K, Jukes H, Kunsagi Z, Schwarz C. The use of aminopenicillins in animals within the EU, emergence of resistance in bacteria of animal and human origin and its possible impact on animal and human health. *J. Antimicrob. Chemother.* [Internet]. 2023; 78(8):1827-1842. doi: <https://doi.org/gshdsz>