



## RESEARCH ARTICLE

### Antimicrobial Susceptibility and Resistance Mechanism of *Campylobacter jejuni* and *Campylobacter coli* isolated from Broiler, Turkey and Cattle in Türkiye

Sahin Çakır<sup>1\*</sup>, Kadir Serdar Diker<sup>2</sup>, Mehmet Akan<sup>3</sup>, Mustafa Bebek<sup>4</sup>, Muammer Göncüoğlu<sup>5</sup>, Şükrü Kırkan<sup>2</sup>, Gonca Öztap Özen<sup>4</sup>, Güzin Şahin<sup>4</sup>, Erkan Taçbaş<sup>6</sup>, Hafize Tuğba Yüksel Dolgun<sup>2</sup>, Hamit Kaan Müştak<sup>3</sup>, İnci Başak Müştak<sup>3</sup>, Özlem Kardoğan<sup>7</sup>, Fethiye Çöven<sup>8</sup>, İrem Gülaçtı<sup>9</sup>, Kamile Kesler<sup>10</sup>, Nevin Turut<sup>11</sup>, Orbay Sayı<sup>12</sup>, Şenay Seyitoğlu<sup>12</sup>, Özge Yılmaz Çağırğan<sup>8</sup>, Veli Gülyaz<sup>13</sup>, Naim Deniz Ayaz<sup>14</sup>, Serap Savaşan<sup>2</sup>, Güzin İplikçioğlu Aral<sup>5</sup>, Muharrem Satılmış<sup>15</sup>, Seza Eskiizmirliiler<sup>8</sup>, Ayhan Akman<sup>16</sup>, Erdem Saka<sup>16</sup>, Arzu Ürşen Aşyemez<sup>17</sup>, Mehmet Hakan Tabak<sup>9</sup>, Durmuş Kahveci<sup>4</sup>, Halim Kavak<sup>4</sup>, Mustafa Ulucan<sup>18</sup>, Seden Arzu Birinci<sup>19</sup>, Hülya Sökmen<sup>20</sup>, Mehmet Levent Kaynar<sup>10</sup>, Asuman Soysal Sarışahin<sup>19</sup> and Harun Hasdoğan<sup>9</sup>

<sup>1</sup>Department of Microbiology, Faculty of Veterinary Medicine, Necmettin Erbakan University, Konya, 42310, Türkiye; <sup>2</sup>Department of Microbiology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın, 09020, Türkiye; <sup>3</sup>Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara, 06070, Türkiye; <sup>4</sup>General Directorate of Food and Control, Ministry of Agriculture and Forestry, Ankara, 06800, Türkiye; <sup>5</sup>Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ankara University, Ankara, 06070, Türkiye; <sup>6</sup>General Directorate of Agricultural Research and Policies, Ministry of Agriculture and Forestry, Ankara, 06800, Türkiye; <sup>7</sup>Veterinary Control Central Research Institute, Ministry of Agriculture and Forestry, Ankara, 06220, Türkiye; <sup>8</sup>Bornova Veterinary Control Institute, Ministry of Agriculture and Forestry, İzmir, 35040, Türkiye; <sup>9</sup>Pendik Veterinary Control Institute, Ministry of Agriculture and Forestry, İstanbul, 34890, Türkiye; <sup>10</sup>Konya Veterinary Control Institute, Ministry of Agriculture and Forestry, Konya, 42090, Türkiye; <sup>11</sup>Adana Veterinary Control Institute, Ministry of Agriculture and Forestry, Adana, 01330, Türkiye; <sup>12</sup>Erzurum Veterinary Control Institute, Ministry of Agriculture and Forestry, Erzurum, 25070, Türkiye; <sup>13</sup>Department of Virology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, 63200, Türkiye; <sup>14</sup>Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, 71450, Türkiye; <sup>15</sup>Menemen Vocational School, Bakırçay University, İzmir, 35665, Türkiye; <sup>16</sup>Samsun Veterinary Control Institute, Ministry of Agriculture and Forestry, Samsun, 55200, Türkiye; <sup>17</sup>Food and Feed Control Central Research Institute, Ministry of Agriculture and Forestry, Bursa, 16160, Türkiye; <sup>18</sup>İskilip District Directorate of Agriculture and Forestry, Ministry of Agriculture and Forestry, Çorum, 19400, Türkiye; <sup>19</sup>Ankara Food Control Laboratory Directorate, Ministry of Agriculture and Forestry, Ankara, 06170, Türkiye; <sup>20</sup>İzmir Food Control Laboratory Directorate, Ministry of Agriculture and Forestry, İzmir, 35200, Türkiye

\*Corresponding author: [sahin.cakir@erbakan.edu.tr](mailto:sahin.cakir@erbakan.edu.tr)

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#### ABSTRACT

Campylobacteriosis is a leading zoonotic disease of global public health significance. Antibiotics are still the scientific and realistic way in the treatment of infectious bacterial diseases. However, AMR has been rapidly increasing worldwide due to overuse and misuse of antibiotics. In this study, *C. jejuni* and *C. coli* strains isolated from cecal samples of broiler, turkey, and cattle at the EPU level were used. The isolates were tested for susceptibility to critical and indicator antimicrobials based on ECOFF, and resistance mechanisms were analysed. Resistance rates according to species for nalidixic acid, ciprofloxacin, and tetracycline among the analysed *Campylobacter* isolates were identified as 75.9, 77.9, and 76.8% in broiler isolates; 80.7, 82.2, and 82.2% in turkey isolates; and 62.9, 63.8, and 75.2% in cattle isolates, respectively. In most strains, FQ resistance was primarily associated with a Thr-86-Ala mutation in the *gyrA* gene, while tetracycline resistance was linked to the *tetO* gene. Single point mutation in 23S rRNA gene (usually A2075G) or *ermB* gene was detected in a few strains with erythromycin resistance. In MDR strains, the *cmeB* membrane efflux gene was identified. In this study, the level of AMR and resistance mechanisms in indicator *C. jejuni* and *C. coli* strains isolated from food-producing animals demonstrated the need for urgent and sustainable collaborative action to monitor and control AMR. This study aimed to characterize the national antimicrobial resistance profiles of *Campylobacter* isolates from broilers, turkeys, and cattle, and to elucidate the underlying resistance mechanisms within a One Health framework.

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## INTRODUCTION

*Campylobacter*, a foodborne, bacterial, and zoonotic pathogen, is one of the leading causes of gastroenteritis in humans (Findik *et al.*, 2001; Shen *et al.*, 2018; Hanafy *et al.*, 2022). *Campylobacter* infections are a significant public health problem worldwide (Tang *et al.*, 2017a; Quino *et al.*, 2022; Nwankwo *et al.*, 2023). It is estimated that *Campylobacter* spp. cause between 400 and 500 million cases of diarrhea annually worldwide (Yao *et al.*, 2016; Tang *et al.*, 2017b; Ma *et al.*, 2021; Veltcheva *et al.*, 2022). In the European Union (EU), Campylobacteriosis has been the most frequently reported gastrointestinal infection in humans since 2005, accounting for 66.8% of all confirmed zoonotic human diseases in 2019 (Liao *et al.*, 2022). Most *Campylobacter* infections in humans are caused by *Campylobacter jejuni* (*C. jejuni*) (90-92%), while *Campylobacter coli* (*C. coli*) accounts for a smaller percentage (7-9%) (Iovine, 2013; Whitehouse *et al.*, 2018; Devi *et al.*, 2019; Ma *et al.*, 2021; Mughini-Gras *et al.*, 2021; Chibwe *et al.*, 2022; Gharbi *et al.*, 2022; Veltcheva *et al.*, 2022).

*Campylobacter* is a major foodborne bacterial pathogen that can be transmitted from animals to humans through the food chain (Hsu *et al.*, 2020; Wang *et al.*, 2022). Transmission to humans occurs via the consumption of raw or undercooked meat and meat products (mainly chicken meat), unpasteurized milk and dairy products, cross-contaminated foods, direct contact with the case, contaminated water, and environmental reservoirs. In healthy individuals, Campylobacteriosis usually presents with an acute and self-limiting infection associated with symptoms of fever, abdominal cramps and watery/bloody diarrhea lasting a few days. However, children, the elderly, pregnant women and immunosuppressed individuals may develop prolonged and more severe symptoms requiring antimicrobial treatment (Şahin *et al.*, 2012; Kayman *et al.*, 2019; Abraham *et al.*, 2020; Hsu *et al.*, 2020; Poudel *et al.*, 2022; Wanja *et al.*, 2023).

When treatment is necessary in humans, macrolides and fluoroquinolones (FQ) are the preferred antimicrobial agents. Tetracyclines and aminoglycosides are alternative treatment options (Shen *et al.*, 2018; Kayman *et al.*, 2019; Quino *et al.*, 2022). However, the worldwide spread of resistance to antibiotics, especially FQ, threatens the effectiveness of antimicrobial treatments (Goulart *et al.*, 2022). For this reason, both the World Health Organisation (WHO) and the United States of America (USA), Centre for Disease Control and Prevention (CDC) have identified antibiotic-resistant *Campylobacter* as a high priority, serious threat to public health (Tang *et al.*, 2017b; Luo *et al.*, 2022; Zhang *et al.*, 2023). Since *C. jejuni* and *C. coli* are zoonotic pathogens, the rising antimicrobial resistance (AMR) among animal reservoirs, which can be transferred to humans via horizontal gene transfer over time, poses a serious risk to Campylobacteriosis treatment.

Consequently, AMR emerging at the human-animal-environment interface presents a global "One Health" challenge (Chibwe *et al.*, 2022).

AMR is one of the complex, urgent and most important health threats facing humanity currently and, in the future. According to WHO's 2019 report, at least 700,000 people die every year in the world due to AMR. Unless action is taken globally, spending \$3-4 billion per year, it is estimated that AMR-related deaths could reach 10 million annually by 2050, at a total cost to the global economy of \$100 trillion (O'Neill, 2016; Anderson *et al.*, 2019; Mancuso *et al.*, 2021; Uddin *et al.*, 2021). The world is rapidly moving towards the post-antibiotic era, where widespread infections could once again lead to deaths in the world if global and urgent action is not taken (WHO, 2015). The United Nations (UN) Food and Agriculture Organisation (FAO), WHO and the World Organisation for Animal Health (WOAH) have together established a tripartite framework that monitors countries' progress in developing policies to combat AMR. International and national efforts to combat AMR have increased in recent years. Examples of international efforts include the WHO's 2015 request to all countries to prepare National Action Plans (NAPs) under the AMR Global Action Plan by 2017, and the UN General Assembly's 2016 political declaration on AMR in which countries committed to work at national, regional and global levels to develop and implement multi-sectoral NAPs in accordance with the "One Health" approach (Anderson and Mossialos, 2020).

In this context, the "National Veterinary Antibiotic Resistance Monitoring Project" was carried out between 2017 and 2021 in cooperation with the Ministry of Agriculture and Forestry (MoAF), Aydın Adnan Menderes and Ankara University, in order to provide data for Türkiye's NAP, to see and monitor the status of veterinary AMR in Türkiye in terms of public health and to create an infrastructure for future actions. The field and laboratory studies of the project were based on directives and guidelines published by EU Commissions such as the European Food Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST). The main approach of the project was to determine the antimicrobial susceptibility of enteric bacteria found in food producing animals in Türkiye and to investigate their resistance mechanisms. The aim of this study was to test the susceptibility of broiler, turkey and cattle *C. jejuni* and *C. coli* isolates to critical and indicator antimicrobials according to Epidemiological Cut-Off Values (ECOFF) at Epidemiological Unit (EPU) level and to examine the resistance mechanisms by using some of the national data obtained from this project.

## MATERIALS AND METHODS

**Sample size, sources, and comparative analysis:** Broiler sampling was conducted from 2018-2019 (Phase I), cattle

sampling from 2019-2020 (Phase II), then broiler and turkey sampling from 2020-2021 (Phase III). A total of 680 broiler cecal samples from 402 EPU in 19 different provinces of Türkiye, 135 turkey cecal samples from 11 different provinces, and 105 cattle samples from 14 different provinces were collected and used in the study. In this study, data were generated in accordance with EU criteria and were evaluated in comparison with reference data reported by EU institutions, including the ECDC, EUCAST, and EFSA.

**Isolation and identification:** The classical culture method specified in ISO10272-1:2006 (<https://www.iso.org/standard/37091.html>) was used for the isolation and identification of *C. jejuni* and *C. coli*. The collected samples were enriched in Preston broth and/or Bolton broth under microaerophilic conditions at 41.5°C for 24 hours. They were then inoculated onto Modified Charcoal Cefoperazone Deoxycholate Agar and incubated under microaerophilic conditions at 41.5°C for 48-72 hours. Following incubation, selected typical colonies were transferred to Columbia Blood Agar supplemented with 5% defibrinated sheep blood. After further incubation under microaerophilic conditions, Gram staining, catalase, oxidase, H<sub>2</sub>S production, and motility tests in Brucella broth were performed. Isolates were tested for Hippurate hydrolysis activity. Additionally, PCR method was conducted for the typing of the isolates for this purpose (Stucki *et al.*, 1995; Gonzalez *et al.*, 1997).

**Antibiotic susceptibility testing:** The antibiotics included in the scope of the project were chosen to represent certain classes of antibiotics, considering their critical importance in treatment, their critical levels in resistance development and their widespread use. *C. jejuni* American Type Culture Collection 33560 was used as the control strain in all studies. The antibiotics analysed for *C. jejuni* and *C. coli* were Gentamicin (GEN), Streptomycin (STR), Nalidixic acid (NAL), Ciprofloxacin (CIP), Erythromycin (ERY), and Tetracycline (TET).

**Disk diffusion method:** For this purpose, the isolated strains were transferred into Brain Heart Infusion Broth and incubated at 37°C until the turbidity reached 0.5 McFarland standard. After adjusting turbidity, a sterile swab was dipped into the culture, excess liquid was drained against the tube wall, and the suspension was spread evenly on the surface of Mueller-Hinton Agar using the swab technique. After the agar surface was dried, antibiotic discs were placed and kept at room temperature for 15 minutes and incubated at 35°C for 24 hours. The evaluation of resistant, intermediate resistant and susceptible was performed according to the ECOFF values defined by EUCAST. Resistance to at least 3 antibiotics from different chemical groups was considered as multidrug resistance (MDR). If no resistance to any of the antibiotics analysed was detected, it was considered as complete susceptibility (CS). Ciprofloxacin-erythromycin (CER) combinations were also recorded.

**Microdilution method:** ISO20776-1:2006 (<https://www.iso.org/standard/41630.html>) method was applied to measure the amount of resistance to the

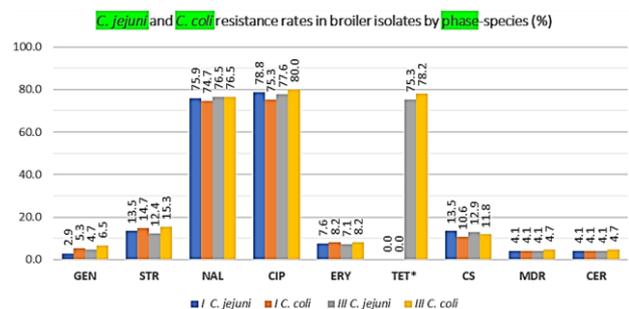
antimicrobial agents. Two-fold dilutions were prepared from the antibiotic stock solution dissolved in the appropriate solvent in the appropriate liquid medium according to the microplate bacteria. Each dilution was inoculated equally from fresh liquid culture at standard density. The plates were incubated under aerobic conditions at 35-37°C for 24 hours. The last dilution in which no growth was observed was considered as Minimal Inhibition Concentration (MIC). The evaluation of resistant or susceptible according to MIC was performed according to the ECOFF values specified by EUCAST.

**Resistance mechanisms:** Resistance-associated genes and mechanisms in *C. jejuni* and *C. coli* were investigated using molecular methods. FQ resistance was analysed by PCR amplification of the *gyrA* gene to detect chromosomal mutations, followed by DNA sequencing for the identification of mutation sites. Tetracycline resistance was determined by PCR detection of the ribosomal protection gene *tetO*. Erythromycin resistance mediated by chromosomal mutations was evaluated by analysing mutations in the 1024-1025 base region of the 23S rRNA gene using PCR and sequencing. The presence of the transferable erythromycin resistance gene *ermB* was detected by PCR. In addition, the membrane-associated efflux gene *cmeB* was identified using PCR (Zirnstein *et al.*, 1999; Jensen and Aarestrup, 2001; Gibreel *et al.*, 2004; Alonso *et al.*, 2005; Jesse *et al.*, 2006; El-Adawy *et al.*, 2012; Han *et al.*, 2012; Obeng *et al.*, 2012; Qin *et al.*, 2014; Wang *et al.*, 2014; Changkwanyeeun *et al.*, 2016; Sierra-Arguello *et al.*, 2016; Zhou *et al.*, 2016; Wozniak-Biel *et al.*, 2018; Gahamanyi *et al.*, 2021).

## RESULTS

### Antimicrobial susceptibility of *Campylobacter* isolates

**Broiler *C. jejuni* and *C. coli* strains:** Ciprofloxacin, nalidixic acid, and tetracycline exhibited the highest resistance rates among EUCAST-selected antibiotics in broiler cecal isolates. In *C. jejuni*, resistance rates were 76.2% for nalidixic acid, 78.2% for ciprofloxacin, and 75.3% for tetracycline. Similarly, in *C. coli*, resistance to nalidixic acid, ciprofloxacin, and tetracycline was observed at rates of 75.6, 77.6, and 78.2%, respectively (Table 1). Due to the absence of phase I data for tetracycline resistance, phase III data were used for the calculations. CS was observed in 13.2% of *C. jejuni* and 11.2% of *C. coli* isolates, while MDR and CER were detected in 4.1% of *C. jejuni* and 4.4% of *C. coli* isolates. Resistance rates for the remaining antibiotics, stratified by phase and species, are presented in Fig. 1.



**Fig. 1:** Antibiotic resistance percentages of *C. jejuni* and *C. coli* isolates of broiler origin resistant to EUCAST selected antibiotics (\*TET: No data for phase I).

**Table 1:** Number of strains resistant to EUCAST selected antibiotics in phase I and III *C. jejuni* and *C. coli* broiler cecal isolates.

Phase	Species	Number	GEN	STR	NAL	CIP	ERY	TET	CS	MDR	CER
I	<i>C. jejuni</i>	170	5	23	129	134	13	nd*	23	7	7
I	<i>C. coli</i>	170	9	25	127	128	14	nd*	18	7	7
III	<i>C. jejuni</i>	170	8	21	130	132	12	128	22	7	7
III	<i>C. coli</i>	170	11	26	130	136	14	133	20	8	8

\*TET: No data for the phase I. (nd: no data). \*\*GEN: Gentamicin; STR: Streptomycin; NAL: Nalidixic acid; CIP: Ciprofloxacin; ERY: Erythromycin; TET: Tetracycline; CS: Completely susceptible; MDR: Multidrug resistant; CER: Ciprofloxacin +erythromycin resistant.

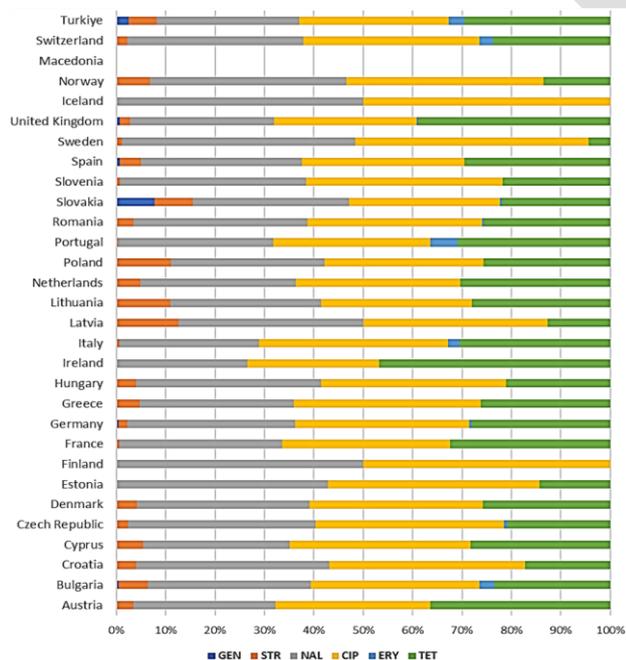
At the EPU level, resistance in *C. jejuni* was observed at rates of 74.1% for nalidixic acid, 76.1% for ciprofloxacin, and 75.6% for tetracycline. In *C. coli*, resistance rates were 72.1% for nalidixic acid, 73.1% for ciprofloxacin, and 80.0% for tetracycline (Table 2).

**Table 2:** Number of broiler EPUs with *C. jejuni* and *C. coli* resistant to EUCAST selected antibiotics in the I and III phases of the study.

Phas	Specie	EPU	GE	ST	NA	CI	ER	TE	C	MD	CE
e	s	Number	N	R	L	P	Y	T	S	R	R
I	<i>C. jejuni</i>	111	2	16	81	83	7	nd*	12	5	4
I	<i>C. coli</i>	111	4	17	78	80	8	nd*	10	6	5
III	<i>C. jejuni</i>	90	2	14	68	70	6	68	10	4	3
III	<i>C. coli</i>	90	4	15	67	67	5	72	9	6	6

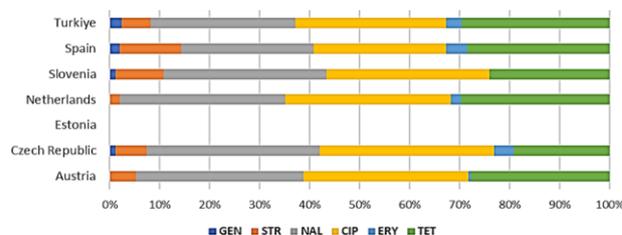
\*TET: No data for the phase I. (nd: no data)

The AMR rates of *C. jejuni* strains analysed in this study were compared with data from official monitoring programmes of EU countries. Overall, resistance rates for the analysed antibiotics were comparable to those reported in most EU countries, with lower resistance levels observed only in Scandinavian countries. High resistance to FQ (nalidixic acid, ciprofloxacin) and tetracycline was commonly reported across EU countries, whereas streptomycin resistance remained relatively low. Erythromycin resistance observed in Türkiye was also detected in a limited number of EU countries, while gentamicin resistance at levels comparable to Türkiye was reported in only one country (Fig. 2).



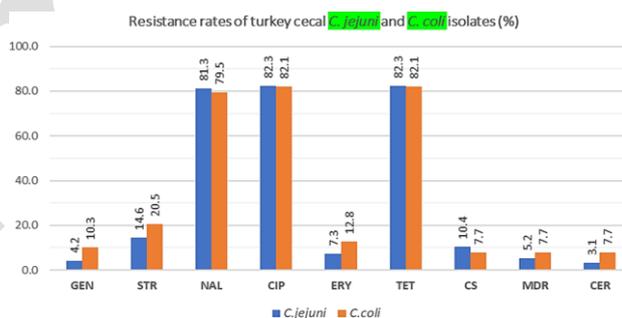
**Fig. 2:** Comparison of percentage cumulative antimicrobial resistance levels of broiler *C. jejuni* isolates with EU countries (EFSA-ECDC, 2020; EFSA-ECDC, 2021).

The AMR rates of broiler-derived *C. coli* strains were compared with cumulative resistance data reported in EU countries monitoring programmes (Fig. 3). Only five countries provided valid monitoring data for broiler-origin *C. coli*. The cumulative resistance levels observed in this study were comparable to those reported by EU countries, like the findings for broiler-derived *C. jejuni* strains.



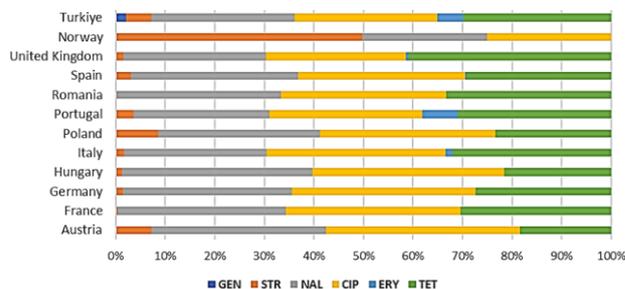
**Fig. 3:** Comparison of percentage cumulative antimicrobial resistance levels of broiler *C. coli* isolates with EU countries (EFS-ECDC, 2020; EFS-ECDC, 2021).

**Turkey *C. jejuni* and *C. coli* strains:** Since *Campylobacter* isolates could not be obtained in the phase I of the project, this part was carried out with *C. jejuni* and *C. coli* isolates in the phase III. In *C. jejuni*, resistance rates to nalidixic acid, ciprofloxacin, and tetracycline were 81.3, 82.3, and 82.3%, respectively. Similarly, in *C. coli*, resistance to nalidixic acid, ciprofloxacin, and tetracycline was observed at rates of 79.5, 82.1, and 82.1%, respectively (Fig. 4). Overall, resistance to EUCAST-selected antibiotics in turkey cecal isolates was high, with the highest rates observed for nalidixic acid, ciprofloxacin, and tetracycline.



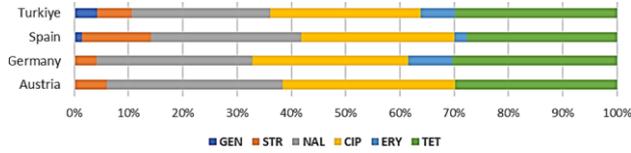
**Fig. 4:** Antibiotic resistance percentages of *C. jejuni* and *C. coli* isolates of turkey origin from 2020-2021.

It was determined that the total AMR was relatively high in turkey *C. jejuni* strains, but the overall resistance trend was similar (Fig. 5). The AMR rates of the analysed turkey *C. coli* strains were found to be at a similar level to the resistance rates in the monitoring programs of EU countries (Fig. 6).

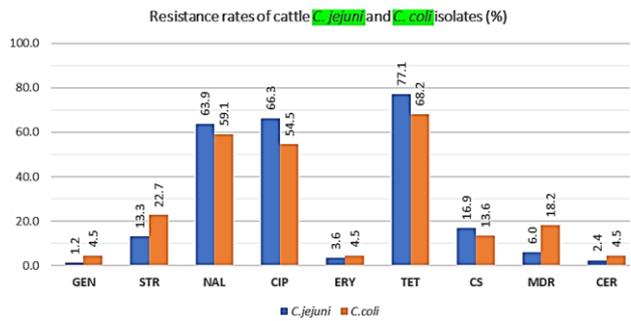


**Fig. 5:** Comparison of percentage cumulative antimicrobial resistance levels of turkey *C. jejuni* isolates with EU countries (EFSA-ECDC, 2020; EFSA-ECDC, 2021).

**Cattle *C. jejuni* and *C. coli* strains:** The highest resistance rates to antibiotics selected by EUCAST in cattle isolates were observed for nalidixic acid, ciprofloxacin, and tetracycline. In *C. jejuni*, resistance to nalidixic acid, ciprofloxacin, and tetracycline was 63.9, 66.3, and 77.1%, respectively. In *C. coli*, resistance to nalidixic acid, ciprofloxacin, and tetracycline was detected at rates of 59.1, 54.5, and 68.2%, respectively (Fig. 7).



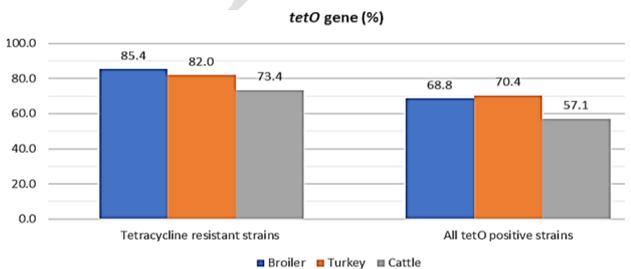
**Fig. 6:** Comparison of percentage cumulative antimicrobial resistance levels of turkey *C. coli* isolates with EU countries (EFSA-ECDC, 2020; EFSA-ECDC, 2021).



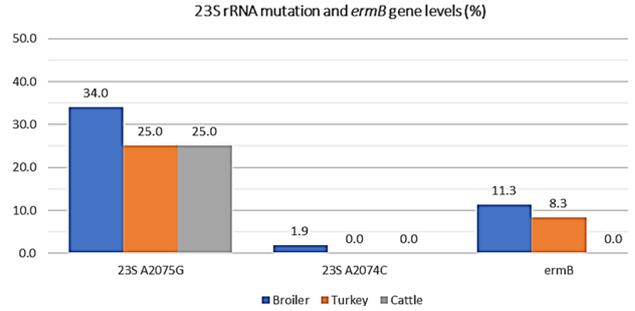
**Fig. 7:** Antibiotic resistance percentages of *C. jejuni* and *C. coli* isolates of cattle origin from 2019-2020.

**Resistance mechanisms in *Campylobacter* strains**

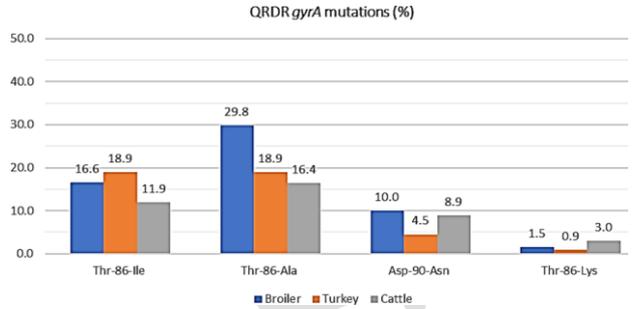
**Broiler *C. jejuni* and *C. coli* strains:** Tetracycline-resistance in the phase III *C. jejuni* and *C. coli* strains analysed, *tetO* gene was found in most of the resistant broiler strains, while this gene was detected at a low rate in tetracycline-susceptible strains. In both species, the *tetO* gene in the phase III was found to be 85.4% in resistant strains, 13.9% in susceptible strains and 68.8% in all strains analysed. No significant difference was observed between the two species. It was understood that the most common 23S rRNA gene mutation in erythromycin-resistant strains was A2075G (34%) and was seen at a higher rate in *C. coli*. While the A2074C mutation was detected only in *C. coli* strains, the A2074G mutation was not observed in any strains. The *ermB* gene was found only in resistant strains (11.3%) (Fig. 9) (Table 3). Analysis of the quinolone resistance determining region (QRDR) of the *gyrA* gene in ciprofloxacin-resistant strains revealed Thr-86-Ala (29.8%), Thr-86-Ile (16.6%), Asp-90-Asn (10%), and Thr-86-Lys (1.5%) mutations, with no significant differences between species (Fig.10) (Table 4). The *cmeB* efflux gene was detected in 72.4% of MDR strains and in 13.2% of CS strains (Fig. 11).



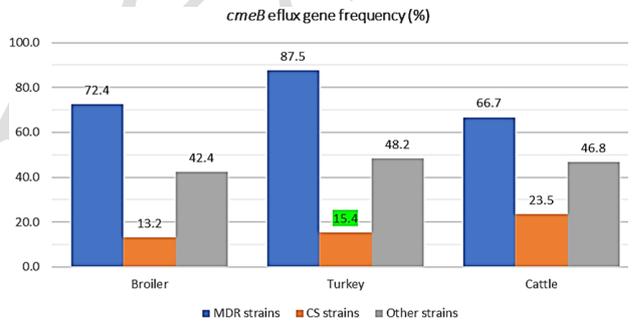
**Fig. 8:** The percentage (%) of *Campylobacter* strains carrying *tetO* gene among tetracycline resistant strains and all strains in different sampled species.



**Fig. 9:** The percentage (%) of 23S rRNA mutations and *ermB* gene carrying among erythromycin resistant *Campylobacter* strains in different sampled species.



**Fig. 10:** The percentage (%) of *gyrA* mutations in ciprofloxacin resistant *Campylobacter* strains in different sampled species.



**Fig. 11:** The percentage (%) of the *cmeB* efflux gene among MDR, CS, and other *Campylobacter* strains in different sampled species.

**Table 3:** Frequency (%) of 23S rRNA gene mutations and *ermB* gene in I and III phase broiler *C. jejuni* and *C. coli* strains and erythromycin resistant strains.

Species/Phase	Erythromycin Resistant Strains (%) In All Strains Analysed (%)						
	A2075G	A2074C	A2074G	ermB	A2075G	A2074C	ermB
<i>C. jejuni</i> /I	30.8	0	0	0	2.4	0	0
<i>C. jejuni</i> /III	25	0	0	8.3	1.8	0	0.6
<i>C. jejuni</i> /I+III	28	0	0	4	2.1	0	0.3
<i>C. coli</i> /I	35.7	7.1	0	14.3	2.9	0.6	1.2
<i>C. coli</i> /III	42.9	0	0	21.4	3.5	0	1.8
<i>C. coli</i> /I+III	39.3	3.6	0	17.9	3.2	0.3	1.5
Total	34	1.9	0	11.3	2.6	0.1	0.9

\*A=adenine, G=guanine, C=cytosine, 2074-2075=base position.

**Turkey *C. jejuni* and *C. coli* strains:** In phase III turkey isolates, the *tetO* gene was detected predominantly in tetracycline-resistant *C. jejuni* and *C. coli* strains, while its presence was low among susceptible strains. Overall, *tetO* was identified in 82% of resistant strains, 16.7% of susceptible strains, and 70.4% of all analysed isolates. The only 23S rRNA mutation associated with erythromycin resistance was A2075G (25%), which was more frequently observed in *C. jejuni*; no A2074C or A2074G mutations were detected (Fig. 9). The *ermB* gene was detected only in resistant *C. coli* strains. When the QRDR *gyrA* gene mutations of ciprofloxacin-resistant *Campylobacter*

**Table 4:** Frequency (%) of QRDR (quinolone resistance determining region) *gyrA* gene mutations in I and III phase broiler *C. jejuni* and *C. coli* strains and ciprofloxacin resistant strains (amino acid/position/amino acid).

Species/Phase	<i>gyrA</i> mutation							
	Ciprofloxacin Resistant Strains (%)				In All Strains Analysed (%)			
	Thr86Ile	Thr86Ala	Asp90Asn	Thr86Lys	Thr86Ile	Thr86Ala	Asp90Asn	Thr86Lys
<i>C. jejuni</i> /I	14.9	29.8	12.7	2.2	11.8	23.5	10	1.8
<i>C. jejuni</i> /III	17.4	34.1	11.4	1.5	13.5	26.5	8.8	1.2
<i>C. jejuni</i> /I+III	16.2	31.9	12	1.9	12.6	25	9.4	1.5
<i>C. coli</i> /I	16.4	26.6	7	0.8	12.4	20	5.3	0.6
<i>C. coli</i> /III	17.6	27.9	8.8	1.5	14.1	22.3	7.1	1.2
<i>C. coli</i> /I+III	17	27.6	7.9	1.1	13.2	21.5	6.2	0.9
Total	16.6	29.8	10	1.5	12.9	23.2	7.8	1.2

strains were analysed, Thr-86-Ala (18.9%), Thr-86-Ile (18.9%), Asp-90-Asn (4.5%) and Thr-86-Lys (0.9%) mutations were found respectively (Fig.10). The *cmeB* efflux gene was detected in 87.5% of MDR strains and in 15.4% of CS strains (Fig.11).

**Cattle *C. jejuni* and *C. coli* strains:** A strong association was observed between tetracycline-resistance and the presence of the *tetO* gene in phase II cattle-derived *C. jejuni* and *C. coli* strains. The *tetO* gene was identified in 73.4% of tetracycline-resistant strains, compared with 7.7% of tetracycline-susceptible strains. In tetracycline-susceptible strains, the *tetO* gene occurred at a low frequency in *C. jejuni* (10.5%) and was not detected in *C. coli*. The only 23S rRNA gene mutation found in erythromycin-resistant cattle strains was A2075G and was detected only in *C. jejuni* (33.3%). The *ermB* gene was not found in any strain. The frequency of *gyrA* gene mutations in ciprofloxacin-resistant strains were observed as Thr-86-Ala (16.4%), Thr-86-Ile (11.9%), Asp-90-Asn (8.9%) and Thr-86-Lys (3%), respectively (Fig. 10). The *cmeB* efflux gene was detected in 66.7% of MDR strains and in 23.5% of CS strains (Fig.11).

**Antimicrobial resistance in animal species:** Comparative analysis revealed host-specific differences in AMR determinants among broilers, turkeys, and cattle. The *tetO* gene was detected less frequently in tetracycline-resistant cattle isolates (73.4%) than in broiler (85.4%) and turkey (82%) *Campylobacter* isolates (Fig. 8). In erythromycin-resistant isolates, 23S rRNA gene mutations were predominantly A2075G and occurred more frequently in poultry; the A2074C mutation was detected only in broilers (1.9%). The *ermB* gene was identified in broiler (11.3%) and turkey (8.3%) isolates but was absent in cattle (Fig. 9). Among ciprofloxacin-resistant isolates, the *gyrA* Thr-86-Ala mutation was most frequent in broilers (29.8%), with similar overall mutation frequencies across host species (Fig. 10). The *cmeB* efflux gene was most prevalent in MDR turkey isolates (87.5%) in Fig. 11.

## DISCUSSION

This study is the first framework research on veterinary AMR conducted in Türkiye. Although the greatest difficulty encountered in the research was the procurement of the necessary isolates, in the end, most of the planned objectives were achieved.

High levels of FQ resistance (75-90%) have been reported in clinical human *Campylobacter* isolates from multiple countries (Dai *et al.*, 2021). Wide-ranging ciprofloxacin resistance levels (30% to >84%) have been

documented among human *Campylobacter* isolates in developing countries (Aleksic *et al.*, 2021). FQ resistance among cattle-derived isolates in the USA reached 35.4% in *C. jejuni* and 74.4% in *C. coli*. High tetracycline resistance rates were reported in *C. jejuni* (88.1%) and *C. coli* (74.8%) (Tang *et al.*, 2017a). In China, FQ resistance was observed in almost all chicken- and pig-derived *C. jejuni* and *C. coli* isolates (Tang *et al.*, 2017b). Resistance to tetracyclines and FQ above 50% has been widely reported across human, animal, and food isolates in the EU (Ocejo *et al.*, 2021). Ciprofloxacin resistance in human *C. jejuni* isolates in Türkiye has been reported at 74.3% and 68.2% (Kayman *et al.*, 2019; Eryildiz *et al.*, 2020). In this research, resistance rates according to species for nalidixic acid, ciprofloxacin, and tetracycline among the analysed *Campylobacter* isolates were identified as 75.9, 77.9, and 76.8% in broiler isolates; 80.7, 82.2, and 82.2% in turkey isolates; and 62.9, 63.8, and 75.2% in cattle isolates, respectively.

Macrolide-resistance is generally <10% in developed countries but markedly higher in developing countries (Tang *et al.*, 2017b; Dai *et al.*, 2021). Macrolide resistance in cattle-derived *C. jejuni* isolates in the USA and Canada is typically reported to be below 10% (Whitehouse *et al.*, 2018). In this study, macrolide resistance was comparable to levels reported in developed countries, except in turkey-derived *C. coli*, where a higher rate (12.8%) was observed.

The *tetO* gene, which is the only tetracycline resistance identified so far in *Campylobacter*, has been reported to be commonly found in *C. jejuni* and *C. coli* (Tang *et al.*, 2017b; Shen *et al.*, 2018). In this study, *tetO* gene was commonly found in tetracycline-resistant strains of broiler, turkey and cattle isolates, while tetracycline-susceptible strains were detected at a low percentage except for the cattle *C. coli* strain.

A novel mechanism of macrolide resistance mediated by the *ermB* gene has recently been revealed in *Campylobacter*. This gene has been detected in *C. jejuni* and *C. coli* strains in China and Europe (Tang *et al.*, 2017b; Quino *et al.*, 2022). In this research, *ermB* gene was found only in erythromycin-resistant broiler *C. jejuni* and *C. coli* strains and only in resistant *C. coli* strains in turkey isolates.

To date, four types of point mutations, A2074C, A2074G, A2074T and A2075G, have been identified in the 23S rRNA gene related to macrolide resistance in *Campylobacter*. It has been reported that A2075G is the most frequently observed of these (Tang *et al.*, 2017b; Aleksic *et al.*, 2021). In this study, consistent with previous reports, A2075G was the most frequently detected 23S rRNA mutation among erythromycin-resistant *Campylobacter* strains.

The most frequently observed mutation in the *gyrA* gene in FQ-resistant *Campylobacter* isolates was Thr-86-

Ile, followed by Asp-90-Asn, Thr-86-Lys, Thr-86-Ala, Thr-86-Val, Asp-90-Tyr and Ala-70-Thr (Tang *et al.*, 2017a; Tang *et al.*, 2017b; Hull *et al.*, 2021). In this study, the predominant *gyrA* QRDR mutation in ciprofloxacin-resistant *Campylobacter* strains was Thr-86-Ala in broiler isolates, whereas Thr-86-Ala and Thr-86-Ile occurred at comparable frequencies in turkey isolates. These patterns are consistent with previous reports.

*cmeB* is the most frequently identified gene and sequence variations have been reported to be associated with an enhanced efflux function (Kassem *et al.*, 2017; Liao *et al.*, 2022). In this study, the *cmeB* efflux gene was detected at high percentages in MDR strains and at relatively low percentages in CS strains across all species. This indicates a direct correlation between the development of resistance and the frequency of the efflux gene.

It has been reported by Whitehouse *et al.* (2018) that *Campylobacter* developed multiple mechanisms for AMR and that these mechanisms may work together synergistically. In this research, the observed resistance rates to FQ and tetracycline, together with the associated resistance mechanisms identified in *Campylobacter* strains, are in agreement with these findings.

Since 2010, legislation on animal health and food safety in Türkiye has been aligned with EU regulations, and the present study was conducted in accordance with criteria established by EU institutions. The high FQ and tetracycline resistance observed in this study, together with the associated resistance mechanisms, could be associated with the long-term, intensive, and sector-specific use of these antimicrobials.

**Conclusions:** This study aimed to characterize the national antimicrobial resistance profiles of *Campylobacter* isolates from broilers, turkeys, and cattle, and to elucidate the underlying resistance mechanisms within a One Health framework. FQ and tetracycline resistance in *C. jejuni* and *C. coli* isolates from broilers and turkeys exceeded established thresholds and were well above the critical levels. Although erythromycin resistance was detected at low frequencies among all isolates, its presence remains notable given its role as the preferred treatment for human *Campylobacter* infections and its generally low resistance rates in most EU countries. A similar pattern was observed for gentamicin. A comparable resistance profile was observed in cattle-derived *Campylobacter* isolates, although resistance rates were relatively lower. Frequent *gyrA* mutations associated with FQ resistance, 23S rRNA mutations and the *ermB* gene linked to erythromycin resistance, the *tetO* gene related to tetracycline resistance, and the presence of the *cmeB* efflux pump collectively demonstrate the involvement of multiple resistance mechanisms.

Measures such as increasing good animal husbandry and good hygiene practices with a holistic “One Health” approach, protection from infections with effective biosecurity measures, prioritization and expansion of vaccination and preventive medicine services, rapid and accurate diagnosis, rational and optimized use of existing antimicrobials, and protection of the environment with good management of animal waste can be listed as measures that will currently limit the AMR burden and spread.

In the long term, researching new antibiotics, new vaccines, different therapeutic and preventive new approaches, encouraging R&D and investments on the subject, conducting modelling on possible emerging threats and establishing early warning systems, raising public awareness through education and training, providing all kinds of financial and political support, and ensuring that all professions and stakeholders with national and global responsibilities act in consensus towards the general goal are urgent priorities. It seems necessary to review the use of antibiotics in certain animal groups and/or situations and to impose limited or complete restrictions on some critically important antibiotic groups.

Acting together by coordinating national and global efforts with an approach that integrates interdisciplinary collaborations to combat this complex and important problem is a must for success. Limiting this complex and important problem, spreading it over time and managing the process well depends on our joining forces.

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