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Original Article
Özgün Araştırma

Resistance to antimicrobials of *Campylobacter jejuni* and *Campylobacter coli* isolated from turkeys in a slaughterhouse

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Abstract: This study measured the percentage of thermotolerant *Campylobacters* (*C. jejuni* and *C. coli)* from samples obtained from a turkey slaughterhouse in Turkey and investigated their antimicrobial resistance to selected antimicrobials by disc diffusion method. Of 28 samples analyzed, 75% were positive for *Campylobacter*. Positivity in turkey cecum samples was 28.5% for *C. coli* and 71.4% for *C. jejuni*. Very high antimicrobial resistance rates were detected for ciprofloxacin and nalidixic acid while resistance to tetracycline was 100% for *C. jejuni* strains. No isolates showed multidrug resistance, or resistance to gentamicin or erythromycin. These results can provide information about the status of *C. coli* and *C. jejuni* resistant to fluoroquinolones, macrolides, aminoglycosides and tetracycline isolated from food animals in Turkey.

Keywords: antimicrobial resistance,*Campylobacter*, Türkiye

Bir mezbahada hindilerden izole edilen *Campylobacter jejuni* ve *Campylobacter coli'* nin antimikrobiyallere direnci

Özet: Bu çalışmada, Türkiye'deki bir hindi mezbahasından alınan numunelerde termofilik *Campylobacter*'lerin (*C. jejuni* ve *C. coli*) yüzdesi ölçülmüş ve disk difüzyon yöntemiyle seçilen antimikrobiyallere karşı dirençleri araştırılmıştır. Analiz edilen 28 örneğin %75'i *Campylobacter* yönünden pozitifti. Hindi sekum örneklerinde %28,5 *C. coli,* %71,4 *C. jejuni* belirlendi. Siprofloksasin ve nalidiksik asite karşı yüksek oranda antimikrobiyal direnç tespit edilirken, *C. jejuni* suşlarında tetrasiklin direnci %100 olarak tespit edildi. Hiçbir izolatta çoklu ilaç direnci, gentamisin veya eritromisine direnç saptanmadı. Bu sonuçlar, Türkiye'de gıda amaçlı kullanılan hayvanlardan izole edilen *C. coli* ve *C. jejuni*'nin florokinolonlara, makrolidlere, aminoglikozidlere ve tetrasikline direnç durumu hakkında bilgi sağlamaktadır.

Anahtar kelimeler: antimikrobiyel direnç, *Campylobacter*, Türkiye

Introduction

The most commonly reported foodborne gastrointestinal infection in humans has been Campylobacteriosis since 2007 (EFSA, 2022). The majority of Campylobacteriosis cases are caused by *Campylobacter jejuni* and *Campylobacter coli*, which cause acute gastroenteritis (Tauxe, 1992; Gillespie et al., 2002; Giacomelli et al., 2014) and, rarely, bacteremia, hepatitis, pancreatitis, and Guillain–Barre syndrome in humans (Butzler, 2004; Louwen et al., 2012). The main reservoir of these *Campylobacter* species is domestic poultry. Campylobacters colonize the cecum of chickens and turkeys (Beery et al., 1988; Sylte et al., 2018; Sylte et al., 2019). Although *Campylobacter* infection often causes gastroenteritis in humans, birds are usually asymptomatic (Wagenaar et al., 2013). Campylobacters are shed in large numbers in poultry feces, which is a major transmission vehicle for *Campylobacter* (Herman et al., 2003). In addition to contaminated food, environmental exposure and direct contact with poultry are associated with infection (Domingues et al., 2012).

Severe and prolonged campylobacteriosis can be treated with antibiotics, with macrolides being the preferred primary antibiotics, although fluoroquinolones, tetracyclines, and gentamycin are also recommended (Aarestrup and Engberg, 2001; Moore et al., 2006; Aarestrup et al., 2008). In serious cases, aminoglycosides are also considered (Aarestrup and Engberg, 2001; Aarestrup et al., 2008; Blaser and Engberg, 2008). However, increased rate of resistance to these antibiotics is a significant public health concern (Blaser and Engberg, 2008), with a report from the Centers for Disease Control and Prevention (CDC) listing drug-resistant *Campylobacter* under "microorganisms with a threat level of

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serious" (Shen et al., 2018). Furthermore, multidrug-resistant strains of *C. jejuni* and *C. coli* from various sources and human infections have also been described (Payot et al., 2004; Luangtongkum et al., 2009; Wieczorek and Osek, 2013; Haldenby et al., 2020). Therefore, especially in the European Union, controlling and monitoring antibiotic resistance in zoonotic pathogens has become mandatory (EFSA, 2020).

In Turkey, no studies have reported the antimicrobial profile of *C. jejuni* and *C. coli* strains isolated from turkeys. Therefore, we used phenotypic methods to investigate the resistance of isolates to fluoroquinolones, macrolides, aminoglycosides and tetracycline.

Materials and methods

Campylobacter jejuni and *Campylobacter coli* isolates

In the current study, 28 turkey cecum samples were analyzed, obtained from slaughterhouses which send samples to our laboratory.

Bacterial culture and DNA extraction

Bacteria isolation was performed according to the ISO 10272-1 (2017) guideline (ISO, 2017).

DNA extraction was performed from bacterial culture on blood agar plates using High Pure PCR Template Preparation Kits (Roche Diagnostics GmbH, Germany) in accordance with manufacturer's instructions.

qPCR Analysis

After cultivation of the bacteria, the plates containing the colonies were stored at 4°C prior to testing their identity with qPCR, which was performed first to determine whether the recovered colonies were *C. jejuni* or *C. coli* using the *Campylobacter coli-jejuni-lari* DNA Test Kit (BioCheck, USA). The qPCR assay was performed using a Rotor-Gene Q (Qiagen Sciences, Germantown, MD) instrument. The qPCR conditions were as follows: 95°C for 3 min followed by 40 cycles at 95°C for 15 s, 60°C for 60 s. Amplification of targets was observed in the FAM, Texas red, Cy-5, and HEX channels for *C. jejuni, C. coli, C. lari*, and internal control, respectively.

A total of 12.5 µL of Mastermix (Bioeksen R&D Technologies Ltd, Turkey), 7.5 µL primer/probe mix with internal control, and 5 µL of template nucleic acid were used.

Antimicrobial susceptibility testing

The antibiotic susceptibilities of the *C. jejuni* and *C. coli* isolates to gentamicin (CN, 10 µg), erythromycin (EM, 15 µg), nalidixic acid (NA, 30 µg), enrofloxacin (EF, 5 µg), ciprofloxacin (Cl, 5 µg), and tetracycline (TE, 30 µg) were determined using the disk diffusion test (Bauer et al., 1966). The test results were evaluated using the criteria published by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2022). Briefly, 0.5 McFarland of the bacterial cultures was prepared then inoculated on Mueller Hinton agar (MHA) (Merck, Germany) supplemented with 5% defibrinated sheep blood. The strains were considered multidrug resistant showing resistance to three or more antimicrobial classes (Giacomelli et al., 2014). The reference strains ATCC 33291 and ATCC 33559 were used as test controls for *C. jejuni* and *C. coli*, respectively.

Results

Identification of *Campylobacter jejuni* and *Campylobacter coli* isolates

Campylobacter spp. were isolated from the cecum samples of all 28 turkeys. The samples revealed that 21 birds were *Campylobacter* positive. Of the 21 *Campylobacter* isolates, 6 (28.5%) isolates were identified as *C. coli* and 15 (71.4%) as *C. jejuni*.

All isolates were also identified using qPCR. The results confirmed the qPCR data, recognizing 15 strains as *C. jejuni* and 6 strains as *C. coli*.

Antimicrobial resistance of isolates

Table, which presents the number of isolates showing susceptibility to each antimicrobial drug, shows firstly that all the investigated strains were susceptible to erythromycin and gentamicin, whereas all the strains were resistant to ciprofloxacin and nalidixic acid. Secondly, all *C. jejuni* isolates were resistant to tetracycline, whereas only 16% of *C. coli* isolates were. Thirdly, all *C. coli* and 93% *C. jejuni* isolates were resistant to enrofloxacin. Finally, none of the strains showed multidrug resistance to three or more classes of antimicrobial drugs.

Table. Results of antimicrobial susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli* isolated from turkeys

	***C. jejuni* (no. of isolates/total)**			***C. coli* (no. of isolates/total)**		
Antimicrobial drugs	*S*	*I*	*R*	*S*	*I*	*R*
Erythromycin	15/15	0/15	0/15	6/6	0/6	0/6
Gentamicin	15/15	0/15	0/15	6/6	0/6	0/6
Nalidixic acid	0/15	0/15	15/15	0/6	0/6	6/6
Enrofloxacin	1/15	0/15	14/15	0/6	0/6	6/6
Ciprofloxacin	0/15	0/15	15/15	0/6	0/6	6/6
Tetracycline	0/15	0/15	15/15	5/6	0/6	1/6

Discussion and Conclusion

This study investigated the presence in turkey cecum samples from a slaughterhouse in Turkey of microbial resistance in thermotolerant *Campylobacter*. Domestic poultry are frequently infected with this species, primarily *C. jejuni* and *C. coli* (Sahin et al., 2002; Corry and Atabay, 2001), with *C. jejuni* being generally the most prevalent species of thermotolerant *Campylobacter* isolated from poultry (Rossler et al., 2019). In the present study, *C. jejuni* (15/21) was more prevalent than *C. coli* (6/21) in turkey cecum samples. Similar results have been reported from Turkey and other countries, where *C. jejuni* was the predominant *Campylobacter* species in broiler intestinal tracts, poultry meats, and at the end of slaughter lines (Yucel and Erguler, 2008; Bostan et al., 2009; Giacomelli et al., 2014; Ozbey and Tasdemi, 2014; Schreyer et al., 2022).

The development of antimicrobial resistance in thermotolerant *Campylobacter*, possibly due to widespread and overuse of antibiotics in animal husbandry, is a matter of great concern (Tadesse et al., 2011; Haldenby et al., 2020). Regardless of the source of *Campylobacter* spp., previous studies have shown that *C. coli* isolates have a higher prevalence of antimicrobial resistance to most antimicrobials than *C. jejuni* (Signorini et al., 2018; Schreyer et al., 2022). In the present study, *C. jejuni* and *C. coli* isolates were equally resistant to nalidixic acid and ciprofloxacin. However, *C. jejuni* isolates were more resistant to tetracycline, whereas *C. coli* isolates were more resistant to enrofloxacin (Table).

In Turkey, ciprofloxacin resistance has been reported in 25% (Yucel and Erguler, 2008) and 74.2% (Cokal et al., 2009) of *C. jejuni* isolates, and in 65.5% (Cokal et al, 2009) and 78.1% (Savasan et al., 2004) of *C. coli* isolates. According to the EFSA and CDC report for 2019–2020, high resistance rates to ciprofloxacin of *C. jejuni* and *C. coli* (52.4–80.0%) isolated from different sources presented (EFSA, 2002). We found almost 100% ciprofloxacin resistance in both *C. jejuni* and *C. coli*, which is one of the highest percentages reported in Turkey or Europe.

We also found 100% resistance to nalidixic acid in both *C. jejuni* and *C. coli* isolates. Other studies in various countries have also reported high levels of resistance of *Campylobacter* isolates to fluoroquinolones (Maesaar et al., 2016; Raeisi et al., 2017). In addition, EFSA reported that nalidixic acid resistance is common among *Campylobacter* isolates in many European Union countries (EFSA, 2023).

Previous studies of tetracycline resistance in *C. jejuni* and *C. coli* isolates from broilers in Turkey have reported prevalences of 42%-76.3% (Yildirim et al., 2005; Cokal et al., 2009) and 58.1%-55.2% (Yildirim et al., 2005; Cokal et al., 2009), although Erdeger and Diker (1995) found lower resistance rates of 15.3% and 24.2% for *C. jejuni* and *C. coli*, respectively. They also found tetracycline resistance rates in *C. jejuni* and *C. coli* of 100% and 16%, respectively.

All the *Campylobacter* isolates in the present study were susceptible to erythromycin and gentamicin, probably because these antimicrobials are not used in Turkish poultry production. Other studies have reported similar results in Turkey and other countries (Yucel and Erguler, 2008; Abay et al., 2014; Giacomelli et al., 2014; Raeisi et al., 2017; Wozniak-Biel et al., 2018; Schreyer et al., 2022), with all isolates being susceptible to gentamicin and fewer than 5% of strains being resistant to erythromycin.

Several studies have reported multidrug resistance in *Campylobacter* species (Qin et al., 2023). Zhao et al. (2016) identified 13 multidrug resistance profiles in *C. jejuni* and *C. coli* isolates from different types of samples (e.g., humans, chicken and turkey), while Schreyer et al. (2022) reported multidrug resistance in 72% of *C. coli* isolates and 69% of *C. jejuni* isolates. In the present study, however, we did not find multidrug resistance in *C. coli* and *C. jejuni* isolates from turkey samples.

Although the samples size was limited and obtained from a single slaughterhouse, this study showed that the sampled slaughter process line is often contaminated with thermotolerant *Campylobacter*. This represents a risk of infection to humans through inappropriate preparation of poultry meat. Our finding of high resistance rates to fluoroquinolones is also alarming because they are used for treating campylobacteriosis in human medicine. In addition, given our finding of a high prevalence of tetracycline resistance in *C. jejuni*, it is also essential to monitor for tetracycline-resistant *C. coli* because tetracycline is a second-line therapeutic agent in therapy of human campylobacteriosis. Since macrolides are not used in veterinary medicine in Turkey, we found very low resistance rates to these antibiotics. In short, to decrease antibiotic resistance prevalence in Turkey, it is essential to monitor antimicrobial resistance in *Campylobacter* species and ensure appropriate use of antimicrobials in animal-food production.

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