Turk J Vet Anim Sci
28 (2004) 391-397

Research Article

Emergence of Quinolone Resistance among Chicken Isolates of Campylobacter in Turkey

Serap SAVAŞAN
Department of Microbiology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY
Alper ÇİFTÇİ, Kadir Serdar DİKER*
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

Received: 25.12.2002

Abstract: The development and extent of quinolone-resistance in broiler isolates of campylobacters over a 15-year period was investigated. Minimal inhibitory concentrations of enrofloxacin, ciprofloxacin and nalidixic acid for 567 *Campylobacter jejuni* and 233 *Campylobacter coli* strains were determined by the agar dilution method. Of the strains, 180, 420 and 200 were isolated during 1987, 1992 and 2000, respectively. Enrofloxacin or ciprofloxacin resistance was not found in isolates from 1987. The first fluoroquinolone resistance (1.4% to enrofloxacin and 1.2% to ciprofloxacin) appeared in strains isolated in 1992, approximately 2 years after the licensing of enrofloxacin in Turkey. The rate of resistance to nalidixic acid was 5.5% in 1987 and 7.3% in 1992. However, the resistance of campylobacters to quinolones increased dramatically in 2000, with 75.5%, 73% and 94.5% of the strains being resistant to enrofloxacin, ciprofloxacin and nalidixic acid, respectively. Fluoroquinolone resistant strains originated from 7.1% of 28 broiler flocks in 1992, and from 92.9% of 14 flocks in 2000. In total, 18% of *C. jejuni* and 24% of *C. coli* strains were resistant to enrofloxacin, and 17.5% of *C. jejuni* and 22.3% of *C. coli* strains were resistant to ciprofloxacin. A high level of cross-resistance was observed among enrofloxacin, ciprofloxacin and nalidixic acid. It was concluded that the uncontrolled use of fluoroquinolones in animals in Turkey caused the emergence and spread of high level resistance among poultry strains of campylobacters, which may have serious effects on both animal and human health and may be a serious food safety concern.

Key Words: *Campylobacter*, quinolone, resistance, chicken

Türkiye'de Tavuk Kökenli Campylobacter Suşlarında Quinolone Dirençliliğinin Yükselişi

Özet: Onbeş yıllık bir periyotta, broiler kökenli Campylobacter suşlarında quinolone dirençliliğinin gelişimi ve boyutları incelendi. Çalışmada, enrofloxacin, ciprofloxacin ve nalidixic asitin 180'i 1987, 420'si 1992 ve 200'ü 2000 yıllarına ait olan 567 *C. jejuni* ve 233 *C. coli* izolatı için MIC değerleri agar dilüsyon yöntemiyle saptandı. 1987 yılı izolatlarında enrofloxacin veya ciprofloxacin dirençliliği bulunmadı. İlk fluoroquinolone dirençliliği (enrofloxacine % 1,4 ve ciprofloxacine % 1,2) enrofloxacin'in Türkiye'de lisans aldığı 1989 yılından yaklaşık iki yıl sonra, 1992'de izole edilen suşlarda görüldü. Nalidixic asite karşı 1987'de % 5,5 ve 1992'de % 7,3 oranında direnç saptandı. Ancak, 2000 yılı *Campylobacter* suşlarında, enrofloxacin, ciprofloxacin ve nalidixic asite karşı sırasıyla % 75,5, 73 ve 94,5 oranları ile, quinolone dirençliliği dramatik bir artış gösterdi. Fluoroquinolone dirençli suşlar 1992'de 28 broiler işletmesinin % 7,1'inde, 2000'de 14 işletmenin % 92,9'unda belirlendi. Toplamda, *C. jejuni* suşlarının % 18'i ve *C. coli* suşlarının % 24'ü enrofloxacine, *C. jejuni* suşlarının % 17,5'i ve *C. coli* suşlarının % 22,3'ü ciprofloxacine dirençli bulundu. Enrofloxacin, ciprofloxacin ve nalidixic asit arasında yüksek düzeyde çapraz dirençlilik görüldü. Türkiye'de hayvanlarda kontrolsüz fluoroquinolone kullanımının, kanatlı Campylobacter suşlarında yüksek düzeyde direnç oluşumuna ve yayılımına yol açtığı, bunun da gıda güvenliği, beşeri ve veteriner hekimlik açısından olasılıkla ciddi sonuçlar doğuracağı sonucuna varıldı.

Anahtar Sözcükler: *Campylobacter*, quinolone, direnç, tavuk

Introduction

Thermophilic campylobacters, including *Campylobacter jejuni* and *Campylobacter coli* are intestinal commensals of domesticated birds. Very low doses of thermophilic Campylobacter cells are sufficient to colonize chicks, and campylobacters generally enter into a non-pathologic, commensal association within the intestine following colonization (1,2). Previous studies on Campylobacter carriage of commercial broilers have reported prevalence rates of 81-100% (3,4). The

*Corresponding author: e-mail: diker@veterinary.ankara.edu.tr

significance of Campylobacter carriage of poultry relates to the potential infection of consumers, and poultry is established as a significant risk factor in the transmission of campylobacters to humans (5). In consequence, the most frequently identified cause of acute diarrheal infection in humans is now thermophilic campylobacters in many parts of the world.

Quinolones are antibacterial agents that act by inhibiting DNA gyrase and topoisomerase IV in susceptible bacteria (6). In addition to the original non-fluorinated quinolone class, there are more than a dozen fluoroquinolones that are approved for use throughout the world. Fluoroquinolones have been widely used in almost all kinds of infection in human and veterinary medicine because of their wide spectra and high levels of antimicrobial activity (7). They have also been used for the treatment of Campylobacter infections and for the empiric treatment of gastroenteritis (8,9). Despite initial optimism, resistance to fluoroquinolone antibiotics among bacteria has increased significantly since their introduction into medicine and agriculture in the late 1980s. Microbial resistance to fluoroquinolones results from mutations in topoisomerase II (DNA gyrase), topoisomerase IV, and/or the activation of drug efflux pumps (6). In Gram-negative organisms, resistance to fluoroquinolones has been shown to be most frequently associated with alterations in *gyrA*, but high-level resistance involves the acquisition of mutations at multiple loci (6). However, *C. jejuni* and *C. coli* tend to develop resistance to quinolones because, unlike in many other species, a single point mutation in the quinolone resistance determining region of their *gyrA* genes suffices to confer high-level resistance (8-11). As a consequence of the widespread use of quinolones in both humans and food animals, the prevalence of quinolone resistant Campylobacter has increased markedly in many parts of the world in the last decade, with the highest occurrences of 97% in human isolates (12-14) and 99% in broiler isolates (4,15). In Turkey, fluoroquinolones were introduced into veterinary practice in 1989, and no resistance was determined in broiler isolates of campylobacters in 1987 and 1992 (16). The aim of this study was to determine the presence and extent of fluoroquinolone resistance in broiler isolates of campylobacters, since no reports were available on this subject in the last decade in Turkey.

Materials and Methods

Campylobacter strains and culture conditions: All Campylobacter strains were consecutive isolates collected from broilers at the Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, in 1987, 1992 and 2000. Strains of the 1987, 1992 and 2000 periods were isolated from 11, 28 and 14 different flocks, respectively. For the isolation of campylobacters, rectal and carcass samples collected from broilers were directly plated on Skirrow or Preston selective agar plates (Oxoid) and incubated at 37 °C in a microaerobic atmosphere for 48 h. Identification of suspected colonies was performed by conventional biochemical and tolerance tests (17). Campylobacters were stored frozen at –80 °C in glycerol-Brucella broth (Oxoid), and only one strain per sample was kept for further study. In the study, 180 isolates from 1987, 420 isolates from 1992 and 200 isolates from 2000 were used. In the susceptibility test, 567 *C. jejuni* and 233 *C. coli* strains were included. Distribution of *C. jejuni* and *C. coli* strains by year was as follows: 113 and 67 in 1987, 318 and 102 in 1992, and 136 and 64 in 2000.

Antimicrobial susceptibility tests: The MICs of nalidixic acid (Sigma), ciprofloxacin and enrofloxacin (Bayer) for campylobacters were determined by the agar doubling dilution method. Briefly, the strains grown overnight on Columbia agar (Oxoid) containing 5% sheep blood were suspended in sterile saline and adjusted to a turbidity matching a 0.5 McFarland standard. An inoculum of 2 µl containing 10^5 CFU was plated in a single spot on a series of Mueller-Hinton agar (Oxoid) containing two-fold serial dilutions of nalidixic acid, ciprofloxacin or enrofloxacin. The range of antibiotic concentrations tested was 0.03 µg/ml to 256 µg/ml for nalidixic acid, and 0.03 µg/ml to 128 µg/ml for ciprofloxacin and enrofloxacin. The inoculated plates were incubated at 37 °C for 48 h in a microaerobic atmosphere containing 5% O_2, 10% CO_2 and 85% H_2. The MIC was defined as the lowest concentration of the drug that completely inhibited visible growth after incubation. The interpretive breakpoints for the MICs to nalidixic acid were ≤8 µg/ml as susceptible and ≥32 µg/ml as resistant. The interpretive breakpoints for the MICs to ciprofloxacin were ≤1 µg/ml as susceptible and ≥4 µg/ml as resistant (18). For enrofloxacin, Bayer's recommendations were ≤0.5 µg/ml as susceptible and ≥2 µg/ml as resistant. MICs at which 50% and 90% of isolates were inhibited were also determined as MIC_{50} and MIC_{90} values, respectively.

Results

In this study, a total of 800 campylobacters comprising 567 *C. jejuni* and 233 *C. coli* strains were used. Of the strains used 180, 420 and 200 were isolated during 1987, 1992 and 2000, respectively. Overall results of antimicrobial susceptibility tests represented as MIC values of enrofloxacin, ciprofloxacin and nalidixic acid for campylobacters in the 3 different periods are shown in Table 1. In 1987, none of the 180 isolates was resistant to enrofloxacin or ciprofloxacin, as shown in Figure 1. The prevalence of nalidixic acid resistance among Campylobacter strains was also low (5.5%) in that year. In 1992, resistance to enrofloxacin, ciprofloxacin and nalidixic acid was found in 1.4%, 1.2% and 7.3% of the 420 Campylobacter strains tested, respectively. In 2000, the resistance of campylobacters to quinolones increased significantly, with 75.5%, 73% and 94.5% of strains being resistant to enrofloxacin, ciprofloxacin and nalidixic acid, respectively. In this period, for both enrofloxacin and ciprofloxacin, MIC ranges were between 0.25 µg/ml and 128 µg/ml, and the MIC_{90} values were 32 µg/ml. MIC ranges of nalidixic acid were between 64 µg/ml and ≥256 µg/ml, and the MIC_{90} value was ≥256 µg/ml. Fluoroquinolone-resistant strains originated from 2 (7.1%) of 28 flocks in 1992, and 13 (92.9%) of 14 flocks in 2000.

In all instances, the prevalence of resistance to quinolones was slightly higher in *C. coli* strains than in *C. jejuni* strains (Table 2). In total, 18% of 567 *C. jejuni* and 24% of 233 *C. coli* were resistant to enrofloxacin, and 17.5% of *C. jejuni* and 22.3% of *C. coli* strains were resistant to ciprofloxacin. A high level of cross-resistance was observed among enrofloxacin, ciprofloxacin and nalidixic acid when the MIC values of fluoroquinolones were compared with nalidixic acid for 800 Campylobacter strains (Figures 2a and 2b). None of the nalidixic acid

Table 1. Frequency distributions of quinolone MICs for *Campylobacter* isolates obtained in 1987, 1992 and 2000.

		Percentage of isolates for which the MIC (µg/ml) was:													
Year	Quinolone	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
1987	Enrofloxacin	7.8	13.9	36.7	31.1	6.6	3.9	-	-	-	-	-	-	-	-
(n: 180)	Ciprofloxacin	4.4	11.7	9.4	37.8	8.9	5.0	2.8	-	-	-	-	-	-	-
	Nalidixicacid	-	-	-	2.8	4.4	11.1	23.9	25.6	16.1	10.0	4.4	1.7	-	-
1992	Enrofloxacin	6.7	11.2	31.4	37.9	7.1	4.3	1.4	-	-	-	-	-	-	-
(n: 420)	Ciprofloxacin	1.2	2.8	28.3	39.8	18.1	5.5	3.1	1.2	-	-	-	-	-	-
	Nalidixicacid	-	-	-	3.3	6.5	14.8	17.1	25.2	16.9	8.8	4.0	2.1	1.2	-
2000	Enrofloxacin	-	-	-	4.5	8.0	11.5	22.5	28.0	13.0	6.5	3.5	1.5	1.0	-
(n: 200)	Ciprofloxacin	-	-	-	2.0	4.5	9.0	11.5	21.0	26.5	15.0	7.0	2.5	1.0	-
	Nalidixicacid	-	-	-	-	-	-	-	-	-	5.5	20.0	32.5	24.5	17.5

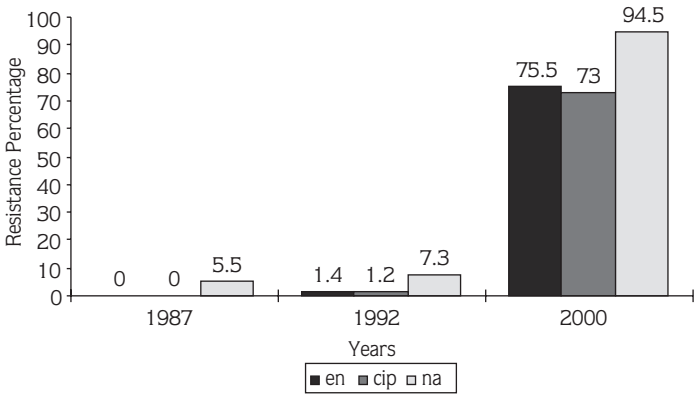

Figure 1. Emergence of quinolone resistance among broiler isolates of campylobacters in Turkey. One hundred and eighty isolates were collected in 1987, 420 isolates in 1992, and 200 isolates in 2000.

Table 2. Comparison of quinolone resistance in *C. jejuni* and *C. coli* isolates. The numbers within the table indicate "number of resistant/number of tested (percentage)".

Quinolone	Year	Resistance (%)	
		C. jejuni	*C. coli*
Enrofloxacin	1987	0/113 (0.0)	0/67 (0.0)
	1992	4/318 (1.3)	2/102 (2.0)
	2000	98/136 (72.1)	54/64 (84.4)
Ciprofloxacin	1987	0/113 (0.0)	0/67 (0.0)
	1992	3/318 (0.9)	2/102 (2.0)
	2000	96/136 (70.6)	50/64 (78.1)
Nalidixic acid	1987	6/113 (5.3)	5/67 (7.5)
	1992	20/318 (6.3)	11/102 (10.8)
	2000	127/136 (93.4)	62/64 (96.9)

susceptible strains showed resistance to enrofloxacin and ciprofloxacin. All ciprofloxacin resistant strains were also resistant to enrofloxacin.

Discussion

Introduction of the fluoroquinolones in the late 1980s provided clinicians with a class of broad-spectrum agents applicable to a range of infections including urinary tract, gastrointestinal, respiratory tract, bone and joint infections, sexually transmitted diseases and infections of the skin and soft tissue (7). The World Health Organization has estimated the annual use of quinolones to be about 120 metric tons, mainly in the United States, the European Union and Japan, and 1.820 tons in China alone (19). During 1997, the use of fluoroquinolones within the European Union has been estimated at 43 tons. The clinical use of fluoroquinolones continues to increase, and they now account for roughly 11% of antimicrobial prescriptions worldwide (20). While information on global use is limited, worldwide use in food animals has been estimated at 120 tons in 1997 (21). In Turkey, the fluoroquinolone enrofloxacin was first licensed for use in veterinary medicine in March 1989, and ciprofloxacin was approved 1 year later for use in human clinical practice. The multiple clinical indications for ciprofloxacin and enrofloxacin, in particular, have led to extensive use of these compounds in many parts of the world, including Turkey, for over a decade. However, widespread use of this class of agents has resulted in an increasing incidence of fluoroquinolone

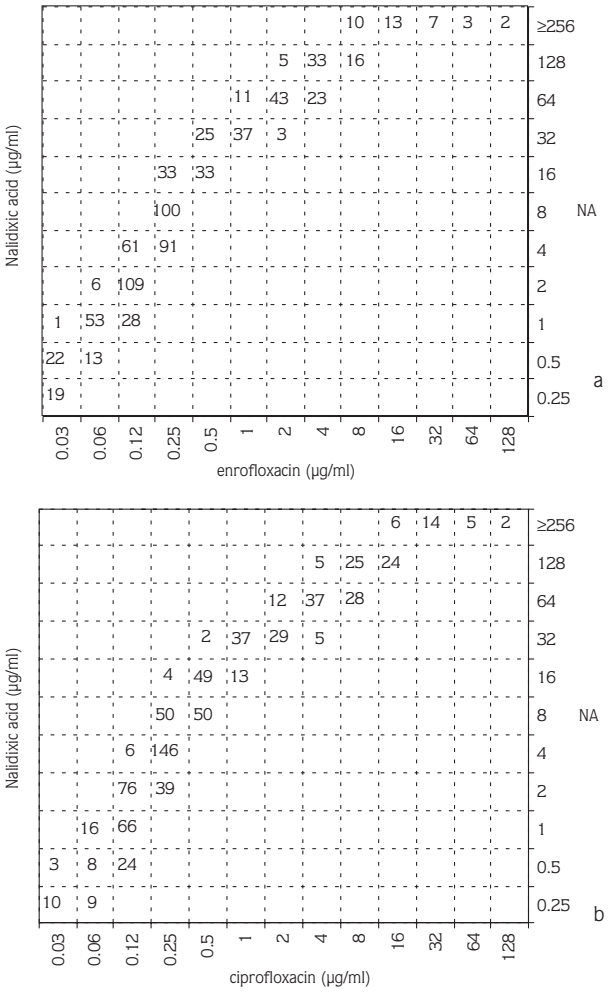

Figure 2. Scattergrams for 800 *Campylobacter* isolates correlating the MICs of nalidixic acid to those of enrofloxacin (a), and ciprofloxacin (b). The numbers within the dashed boxes indicate the numbers of *Campylobacter* isolates.

resistance in many bacteria all over the world (20). The development of clinically significant levels of resistance in animal strains of campylobacters would cause serious problems in human and veterinary medicine, since campylobacteriosis is considered a zoonotic disease, and animals such as poultry may act as reservoirs for campylobacters. In this regard, it was of great importance to ascertain the prevalence of quinolone resistant campylobacters of avian origin in Turkey.

In this study, all Campylobacter isolates collected in 1987 were susceptible to fluoroquinolones, and most strains were susceptible to nalidixic acid. This was a predictable finding, since fluoroquinolones were not in

use in 1987, and nalidixic acid was not a veterinary drug. In other studies conducted prior to the introduction of fluoroquinolones into practice, resistance has not been observed in human or animal isolates of campylobacters in Turkey (16,22). However, the first fluoroquinolone resistant strains (1.4% to enrofloxacin and 1.2% to ciprofloxacin) appeared in 1992, approximately 2 years after the licensing of fluoroquinolones. It was the occurrence of resistance only 2 years after the limited use of fluoroquinolones, that raised the alarm. The first cases of fluoroquinolone resistance with similar rates to those in the current study have been also reported generally in this period from all around the world, particularly in European countries (23,24). The rate of resistance to enrofloxacin rose dramatically from 1.4% in 1992 to 75.5% in 2000 in the present study. To our knowledge, this is one of the highest prevalences of resistance to fluoroquinolones in campylobacters that has been reported in recent years. Similar patterns of resistance development have also been noted in other studies. Ciprofloxacin resistance has been found in 99% of broiler isolates of campylobacters in Spain (4) and 62.1% in Belgium (15). In human isolates, high resistance rates have been reported in Spain (81%)(13), Hong Kong (97%) (14) and Thailand (84%) (12). Among these high resistance rates, it is also possible to see low level resistance to fluoroquinolones (as low as 10%) in occasional studies. These contradictory findings may be caused by prohibited use or later licensing of these drugs in some countries. In this study, we encountered a higher prevalence of resistance to quinolones in *C. coli* than in *C. jejuni*. Prats et al. (13) and Aquino et al. (25) have also reported that *C. coli* is more resistant to quinolones than is *C. jejuni*.

Development of resistance to nonfluorinated (nalidixic acid) and fluorinated (enrofloxacin and ciprofloxacin) quinolones followed the same patterns in all the years considered in the study (Figure 2). The similar proportions and speeds of resistance showed that cross resistance had occurred between nalidixic acid and fluoroquinolones. The level of resistance to enrofloxacin (used in domestic animals) was also the same as the level of resistance to ciprofloxacin (available for human clinical use). This is due to the fact that resistance to one fluoroquinolone generally confers this on the entire class of fluoroquinolone drugs (10,13). This may be explained by the fact that a single mutation in either topoisomerase can confer resistance to both nonfluorinated and fluorinated quinolones in *C. jejuni* or *C. coli* (8,9).

The high levels of quinolone resistance detected in campylobacters in the present study may be caused by several factors. *C. jejuni* and *C. coli* tend to develop resistance to quinolones because, unlike in many other species, a single point mutation in the quinolone resistance-determining region (QRDR) of its *gyrA* gene suffices to confer high-level resistance (8,9). This may explain the significantly higher level of quinolone resistance in Campylobacter when compared with other genera. Exposure of a microorganism to a fluoroquinolone has been shown to be the most significant factor in the selection of resistant mutants. The isolation of resistant mutants of campylobacters in vivo after therapeutic treatment with quinolone has been reported by several authors (26,27). Quinolone treatment of Campylobacter-colonized broiler chickens has been established to induce quinolone resistance under experimental conditions within 5 days (28,29). In Turkey, the addition of antimicrobial agents to feed or water for therapeutic or prophylactic purposes remains uncontrolled, and the volume of drugs used is high. Intensively farmed animals are often treated as a group and are given massive amounts of medication. In addition, quinolones are mostly excreted as unchanged substances and are among the most persistent drugs in the environment (30). Therefore, the discharge of quinolones in the environment may increase the risk of further contact with environmental strains and select resistant mutants among bacterial populations. Since fluoroquinolones are in extensive use for all bacterial infections in Turkey, conditions of high selective pressure have forced the mutant Campylobacter strains to multiply and establish themselves as the dominant population. The abusive and anarchic use of antibiotics is probably the leading factor in the high levels of resistance detected among avian Campylobacter strains in Turkey.

Resistance to quinolones in campylobacters bears on both diagnostic and therapeutic aspects. Nalidixic acid resistance has been used as an important phenotypic marker to distinguish between *C. jejuni*/*C. coli* and *C. fetus* subsp. *fetus* or *C. lari* (17). *C. jejuni*/*C. coli* strains have generally been regarded as susceptible to nalidixic acid, whereas most *C. fetus* subsp. *fetus* and *C. lari* strains are intrinsically resistant to high levels of nalidixic acid. Thus, resistance to quinolones in campylobacters is

regarded as an important tool for their identification. However, increasing resistance (up to 94.5%) of *C. jejuni* and *C. coli* strains to nalidixic acid as detected in this study shows that nalidixic acid resistance should not be used as a criterion in the differentiation of Campylobacter species any more. The high prevalence of quinolone resistance detected in broiler isolates of campylobacters may have important consequences for public health. Human Campylobacter infections usually occur following ingestion of improperly handled or undercooked food, and poultry meat has been considered as the most common source of infection (5). Fluoroquinolones are often recommended to patients who require treatment (7). However, if a person is infected with quinolone resistant campylobacters of poultry origin, this drug will be ineffective. Before fluoroquinolones were introduced into veterinary medicine, they had been widely used in human medicine in a number of countries without the emergence of quinolone resistance. In contrast, emerging quinolone resistance in humans often coincides with or follows the approval of the use of fluoroquinolones in animal husbandry (11). In this study, it was concluded that the use of fluoroquinolones in veterinary medicine caused the emergence and spread of resistance among Campylobacter strains, with potentially serious effects on food safety and on both animal and human health. These findings should reinforce the message that preventing the spread of antibiotic resistance requires the prudent and controlled use of antibiotics, not only in humans but also in veterinary medicine.

References

1. Shane, S.M.: The significance of *Campylobacter jejuni* infection in poultry: a review. Avian Pathol. 1992; 21: 189-213.
2. Hald, B., Knudsen, K., Lind, P., Madsen, M.: Study of the infectivity of saline-stored *Campylobacter jejuni* for day-old chicks. Appl. Environ. Microbiol. 2001; 67: 2388-2392.
3. Yıldız, A., Diker, K.S.: Campylobacter contamination in chicken carcasses. Turk. J. Vet. Anim. Sci. 1992; 16: 433-439.
4. Saenz, Y., Zarazaga, M., Lantero, M., Gastanares, M., Baquero, F., Torres, C.: Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997–1998. Antimicrob. Agents Chemother. 2000; 44: 267-271.
5. Skirrow, M.B.: Epidemiology of Campylobacter enterit. Int. J. Food Microbiol. 1991; 12: 9-16.
6. Hooper, D.C.: Mechanisms of fluoroquinolone resistance. Drug Resist. Updates 1999; 2: 38-55.
7. Hooper, D.C.: Clinical applications of quinolones. Biochim. Biophys. Acta 1998; 1400: 45-61.
8. Piddock, L.J.V.: Quinolone resistance and Campylobacter. Clin. Microbiol. Infect. 1999; 5: 239-243.
9. Engberg, J., Gerner-Smidt, P., Aarestrup, F.M., Taylor, D.E., Nachamkin, I.: Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. 2001; 7: 24-35.
10. Gibreel, A., Sjogren, E., Kaijser, B., Wretlind, B., Skold, O.: Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational changes in *gyrA* and *parC*. Antimicrob. Agents Chemother. 1998; 42: 3276-3278.
11. Ruiz, J., Goni, P., Marco, F., Gallardo, F., Mirelis, B., Jimenez de Anta, M.T., Vila, J.: Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol. Immunol. 1998; 42: 223–226.
12. Hoge, C.W., Gambel, J.M.: Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin. Infect. Dis. 1998; 26: 341–345.
13. Prats, G., Mirelis, B., Llovet, T., Munoz, C., Miro, E., Navarro, F.: Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995–1998 in Barcelona. Antimicrob. Agents Chemother. 2000; 44: 1140–1145.
14. Lau, S.K.P., Woo, P.C.Y., Leung, K.W., Yuen, K.Y.: Emergence of cotrimoxazole- and quinolone-resistant Campylobacter infections in bone marrow transplant recipients. Eur. J. Clin. Microbiol. Infect. Dis. 2002; 21: 127–129.
15. Van Looveren, M., Daube, G., De Zutter, L., Dumont, J.M., Lammens, C., Wijdooghe, M.,Vandamme, P., Jouret, M., Cornelis, M., Goossens, H.: Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium. J. Antimicrob. Chemother. 2001; 48: 235-240.
16. Diker, K.S.: In vitro susceptibility of campylobacters isolated from poultry to enrofloxacin and ciprofloxacin. Acta Gastroenterol. Belgica 1993; 56 (suppl.): 36.
17. Diker, K.S.: Studies on the identification of Campylobacter species isolated from sheep and cattle. Turk. J. Vet. Anim. Sci. 1985; 9: 232-240.
18. National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial susceptibility testing. 8th Informational Suppl. NCCLS document M100-S8. 1998, National Committee for Clinical Laboratory Standards, Wayne, PA.

19. World Health Organization: Use of quinolones in food animals and potential impact on human health. Report of a WHO Meeting. Report WHO/EMC/ZDI/98.10, 1998, World Health Organization, Geneva, Switzerland.

20. Thomson, C.J.: The global epidemiology of resistance to ciprofloxacin and the changing nature of antibiotic resistance: a 10 year perspective. J. Antimicrob. Chemother. 1999; 43(Suppl. A): 31–40.

21. Van Diest, J., De Jong, A.: Overview of quinolone usage for food-producing animals. In: Use of quinolones in food animals and potential impact on human health. Report and Proceedings of a WHO Meeting. Geneva: World Health Organization, 1999; p. 97.

22. Gür, D., Hascelik, G., Akyon, Y., Akalin, H.E., Diker, K.S.: In vitro susceptibility of *Campylobacter jejuni* and *Campylobacter coli* to quinolone antibiotics. Mikrobiyol. Bült. 1989; 23: 185-189.

23. Adler-Mosca, H., Altwegg, M.: Fluoroquinolone resistance in *Campylobacter jejuni* and *C. coli* isolated from human faeces in Switzerland. J. Infect. 1991; 23: 341-342.

24. Sjogren, E., Kaijser, B., Werner, M.: Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. Antimicrob. Agents Chemother. 1992; 36: 2847–2849.

25. Aquino, M.H.C., Filgueiras, A.L.L., Ferreira, M.C.S., Oliveira, S.S., Bastos, M.C., Tibana, A.: Antimicrobial resistance and plasmid profiles of *Campylobacter jejuni* and *C. coli* from human and animal sources. Lett. Appl. Microbiol. 2002; 34: 149-153.

26. Adler Mosca, H., Luthy-Hottenstein, J., Martinetti, G., Burnens, A.P., Altwegg, M.: Development of resistance to quinolones in five patients with campylobacteriosis treated with norfloxacin or ciprofloxacin. Eur. J. Clin. Microbiol. Infect. Dis. 1991; 10: 953-957.

27. Segreti, J., Gootz, T.D., Goodman, L.J., Parkhurst, G.W., Quinn, J.P., Martin, B.A., Trenholme, G.M.: High-level quinolone resistance in clinical isolates of *Campylobacter jejuni*. J. Infect. Dis. 1992; 165: 667-670.

28. Jacobs-Reitsma, W.F., Kan, C.A., Bolder, N.M.: The induction of quinolone resistance in Campylobacter in broilers by quinolone treatment. Lett. Appl. Microbiol. 1994; 19: 228-231.

29. McDermott, P.F., Bodeis, S.M., English, L.L., White, D.G., Walker, R.D., Zhao, S., Simjee, S., Wagner, D.D.: Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. J. Infect. Dis. 2002; 185: 837-840.

30. Halling-Sorensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Lützhoft, H.C.H., Jorgensen, S.E.: Occurrence, fate and effects of pharmaceutical substances in the environment - a review. Chemosphere 1998; 36: 357–393.