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Research Article

Prevalence and Antibiotic Susceptibility of Thermophilic *Campylobacter* Species in Broiler Chickens

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Abstract: The aim of the present study was to investigate the prevalence of thermophilic *Campylobacter* species in broiler chicken faecal samples and on their carcasses. The possible routes of carcass contamination were assessed from slaughterhouse to market. Furthermore, the study aimed to determine the antibiotic susceptibility of *Campylobacter* isolates from broilers.

Thermophilic *Campylobacter* spp. was isolated from 393 (91.8%) of 428 samples examined. A total of 53 out of 57 rectal swab samples was positive for thermophilic *Campylobacter* spp. Thermophilic *Campylobacter* spp. were isolated from 93.6%, 92.9% and 91.3% of broiler carcass, faecal and caecal samples, respectively. No *Campylobacter* was isolated from scalding tank water samples. However, all samples collected from cold water tanks were found to be contaminated with thermophilic *Campylobacter* spp. Overall, 92.2% and 7.8% of the isolates were identified as *Campylobacter jejuni* and *C. coli*. Of the *C. jejuni* isolates from broiler chickens, 30.6% were resistant to β-lactam antibiotics and 31.3% were resistant to quinolone group antibiotics. Of the *C. coli* isolates from broiler chickens, 30.7% were resistant to β-lactam antibiotics and 31.2% were resistant to quinolone group antibiotics.

With these results we conclude that the widespread faecal contamination of broiler carcasses in broiler farms in Kırıkkale with thermophilic *Campylobacter* spp. constitutes a risk for public health. This study once more indicates the application of a preventive system such as HACCP (Hazard Analysis of Critical Control Points) is strongly required in the contamination control of *campylobacter*s in broiler farms and slaughterhouses.

Key Words: Prevalence, antibiotic susceptibility, thermophilic *Campylobacter* spp, broiler chickens

Broiler Tavuklarda Termofilik *Campylobacter* Türlerinin Prevalansı ve Antibiyotik Duyarlılıkları

Özet: Bu çalışmanın amacı, broiler karkas ve dışkı örneklerinde termofilik *Campylobacter* türlerinin prevalansını araştırmaktır. Çalışmada, broyler kesimhanelerinde kesim sırası ve sonrasında karkasların dışkı ve dolayısıyla *Campylobacter*ler ile olası kontaminasyon riski belirlendi. Ayrıca, bu çalışmada, broilerlerden izole edilen *Campylobacter* izolatlarının antibiyotik duyarlılık durumları da araştırıldı.

Broyler kümeslerinden alınan rektal swab örnekleri ve kesimhanelerden alınan karkas swab, barsak (sekum) ve karkas yıkama suyu olmak üzere incelenen toplam 428 adet materyalin 393'ünden (% 91,8) termofilik *Campylobacter* türleri izole edildi. Broyler piliçlerden alınan rektal örneklerden % 92,9 oranında izolasyon gerçekleştirildi. Kesimhanelerden alınan karkas swab örneklerinden % 93,6 oranında, barsak (sekum) örneklerinden ise % 91,3 oranında izolasyon yapıldı. Haşlama tankından alınan karkas yıkama suyu örneklerinden izolasyon yapılamazken, soğuk su tankından alınan örneklerin tamamından (% 100) *Campylobacter*ler izole edildi. En yüksek oranda izolasyon tavuk karkaslarından (% 93,6) ve tavuk dışkılarından (% 92,9) yapıldı. İzole edilen suşların % 92,2'si *Campylobacter jejuni*, % 7,8'i *C. coli* olarak identifiye edildi. *C. jejuni* izolatlarının % 30,6'sı β-laktam antibiyotiklerine ve % 31,3'ü kinolon grubu antibiyotiklere dirençli bulundu. *C. coli* izolatlarının ise % 30,7'si b-laktam antibiyotiklerine ve % 31,2'si ise kinolon grubu antibiyotiklere dirençli bulundu.

Sonuçlar Kırıkkale broyler çiftliklerindeki broyler karkaslarında termofilik *Campylobacter*ler ile yaygın bir fekal kontaminasyonun olduğunu ve bunun halk sağlığı açısından önemli bir risk oluşturduğunu göstermektedir. Bu çalışma, HACCP (Kritik Kontrol Noktalarında Tehlike Analizi) gibi koruyucu bir sistem uygulamasının *campylobacter*lerin broyler çiftlikleri ve kesimhanelerdeki kontaminasyon kontrolleri için gerekliliğini bir kez daha vurgulamaktadır.

Anahtar Sözcükler: Prevalans, antibiyotik duyarlılık, thermofilik *Campylobacter*, broiler tavuk

Introduction

Campylobacteriosis is primarily a zoonotic infection of humans and animal derived foods are significant sources of infection. Campylobacteriosis has been associated with poultry carcasses and further processed poultry products (1,2). The thermophilic *Campylobacter* spp. are among the most frequently reported causes of bacterial enteritis particularly in the developed countries (3).

Elucidation of the nature and diversity of *Campylobacter* spp. contaminating the human food chains and assessing their epidemiological significance is essential for the control of foodborne endemic outbreaks (3).

Epidemiological investigations and Public Health Laboratory reports in many countries show that poultry products are primary sources for campylobacter infections in humans and the thermophilic *Campylobacter* spp. are commonly isolated from poultry faeces and poultry products (3-5). In England and Wales alone, 58,000 cases of human campylobacter infection were reported in 1998, representing a 16% increase over the comparable incidence in 1997 (5). In Denmark, the number of cases has more than tripled during the last 7 years from approximately 22 cases/100,000 inhabitants in the years 1980 to 1992 to 78/100,000 in 1999 (3,4).

A previous study conducted in Canada has reported as 85% *Campylobacter* spp. isolation rate from faecal samples collected from 28 broiler farms (6). Neil et al. (7) found 90% of 12 broiler farms to be positive for *Campylobacter* spp. in Ireland.

Investigations concerning possible *Campylobacter* contamination sources and carrier status of poultry products in Turkey are relatively limited (8-11). To our knowledge, detailed large-scale epidemiological investigations on sources of animal and human *campylobacter* infections have not been conducted yet. Diker and Yardımcı (8) reported that faeces from 7 flocks were positive for thermophilic *campylobacter*s. Baysal and Güler (9) indicated that the thermophilic *Campylobacter* spp. contamination rate was 26% in liver, duodenum, and bile samples collected from broilers. Yıldız and Diker (10) found that the contamination rate of *thermophilic Campylobacter* spp. was 95% in faeces from 2 broiler flocks examined and reported that carcass samples obtained from 4 slaughterhouses were 100% positive. Akan et al (11) shown that the contamination rates for thermophilic *Campylobacter* spp. were 87.2% for the rectal samples and 92% to 100% for carcass samples.

In recent years, however, an increased proportion of *Campylobacter* isolates have been reported to be resistant to antibiotics especially fluoroquinolones (12). There is a growing concern that veterinary use of antimicrobials in food animals can select for resistant *Campylobacter* spp., which may subsequently be transmitted to humans through the food chain (12,13). However, only a few reports (12-14) have examined the current trend of antimicrobial susceptibility in *Campylobacter* isolates from healthy animals or retail meats.

The aim of the present study was to determine the prevalence of thermophilic *Campylobacter* species in chicken faecal and carcass samples. In addition, the possible routes of carcass contamination in the slaughterhouse to market chain were investigated. Furthermore, the study aimed to determine the antibiotic susceptibility of *Campylobacter* isolates obtained from broilers.

Materials and Methods

Sampling procedure

A total of 428 samples from broiler chickens (Ross Breeder) which contained faecal samples and carcass rinse fluid samples were examined. The samples were obtained from 3 different poultry-processing plants and kept cool during transportation (Table 1). Samples from cold and scald tanks were also obtained. Cotton swabs were used to sample carcass surfaces. After sampling, the swabs were put into empty sterile capped tubes and immediately transported to the laboratory and examined for the presence of *Campylobacter* spp. Caecal samples were also taken after evisceration from a processing plant. These samples were individually placed into sterile plastic bags and transported in an insulated cooled container to the laboratory. All samples were examined within 24 h of collection.

Isolation studies

Selective plating

The modified charcoal cefoperazone deoxycholate agar (mCCDA) comprised a commercially supplied charcoal base (Oxoid) and cefoperazone (32 mg/l) (Sigma) was used for the isolation of *Campylobacter* spp.

Table 1. Numbers and sources of samples used for isolation of *Campylobacter* spp. in this study.

Samples	Numbers of samples
Rectal swab	57
Carcass swab	266
Caecum	93
Cold tank wash water	6
Scald tank wash water	6
TOTAL	428

Samples of carcass and faecal swabs were directly streaked onto mCCDA. 0.1 ml of tank water samples was directly streaked onto mCCDA and was filtered through a sterile cellulose acetate membrane filter, with a diameter of 47 mm and a 0.65 µm pore size (Sartorius). Then filters were placed onto mCCDA plate surfaces. All plates were incubated at 37 °C in a hydrogen-enriched microaerobic atmosphere which was obtained by use of a gas generating kit BR056 (Oxoid), (6% O_2, 6% CO_2, 3% H_2, and 85% N_2) without catalyst and examined for suspect colonies after 2 days (15).

Each caecum sample was aseptically submerged into boiling water for about 5 s in laboratory. Approximately 1 g of caecal content was squeezed into 10 ml of maximum recovery diluent CM733 (Oxoid) and shaken vigorously using a vortex mixer for ca 1 min; 10 µl of this suspension was streaked onto mCCDA and inoculated plates were incubated at 37 °C in a microaerobic atmosphere (15).

Identification and phenotypic characterisation of bacterial isolates

All isolates were characterised using the biochemical and tolerance activity procedures recommended by On et al. (16). One suspect colony from each colony-type on each plate was picked, and checked by Gram stain, oxidase, catalase, hippurat, indoxyl acetate and susceptibility to cephalotin and nalidixic acid and microscopic examination. Colonies giving reactions typical for *Campylobacter* spp. were purified by restreaking onto mCCDA. Mueller-Hinton agar with defibrinated 5% sheep blood was used for subcultures and biochemical and tolerance tests.

Antimicrobial agents and susceptibility testing

Campylobacter jejuni NTCC 11168 was used as the QC organism for disc diffusion test. For the disc diffusion testing, nalidixic acid (Oxoid) (30 µg), cephalothin (Oxoid) (30 µg), norfloxacin (Oxoid) (10 µg), erythromycin (Oxoid) (15 µg), amoxycillin (Oxoid) (20 µg), ampicillin (Oxoid) (10 µg), gentamycin (Oxoid) (10 µg) and tetracycline (Oxoid) (30 µg), enrofloxacin (Bayer) (5 µg) and danofloxacin (Pfizer) (5 µg) discs were used.

Disc diffusion tests were performed according to standard procedure (17) on Mueller-Hinton agar CM337 (Oxoid) containing defibrinated 5% sheep blood. Inocula were prepared in Mueller-Hinton broth CM405 (Oxoid) with a density adjusted to 0.5 McFarland. One hundred microlitres of the selected broth cultures were streaked onto Mueller-Hinton agar plates, and then the antibiotic discs were placed. Inoculated plates were incubated at 37 °C in a hydrogen-enriched microaerobic atmosphere as described above and examined after 2 days. After 48 h of incubation, the diameters of the inhibition zones were measured with slipping callipers. National Committee for Clinical Laboratory Standards were used for interpretation of test results (17).

Results

Prevalence and species distribution of *Campylobacter* isolates.

A total of 428 samples were examined, of which 91.8% were positive for thermophilic *Campylobacter* spp. by direct plating and/or filtration culture technique. Isolation rates varied among sources (Table 2), with cold water tank samples having the highest contamination rate (100%), followed by broiler carcasses (93.6%), followed by rectal swab (92.9%) and caecum (91.3%) samples. From the expected species distribution, 92.2% and 7.8% of the isolates were determined as *C. jejuni* and *C. coli*, respectively.

The prevalence of individual thermophilic *Campylobacter* spp. also varied according to the origin of the breed, with *C. jejuni* being the predominant species in broiler chickens.

Antimicrobial susceptibility results

30.6% and 31.3% of the *C. jejuni* isolates were found to be resistant to penicillins and quinolones, respectively (Table 3). 30.7% and 31.2% of the *C. coli* isolates from broiler chickens were found to be resistant to penicillins and quinolone antibiotics, respectively (Table 3).

Table 2. The overall isolation rates and species distribution of the *C. jejuni* and *C. coli* isolates obtained in this study.

Sample type	Numbers of samples	Isolation rate (%)		
		Overall	*C. jejuni*	*C. coli*
Rectal swab	57	53 (92.9)	49 (92.4)	4 (7.6)
Caecum	93	85 (91.3)	78 (91.7)	7 (8.2)
Carcass swab	266	249 (93.6)	230 (92.3)	19 (7.6)
Cold tank wash water	6	6 (100)	5 (83.3)	1 (16.6)
Scald tank wash water	6	0 (0)	0 (0)	0 (0)
TOTAL	428	393 (91.8)	362 (92.2)	31 (7.8)

Table 3. Antibiotic resistance/susceptibility profiles of *C. jejuni* and *C. coli* chicken isolates against various antibiotic agents.

Antimicrobial agent	*C. jejuni*			*C. coli*		
	*n/362	S (%)	R (%)	*n/31	S (%)	R (%)
Cephalothin (30 μg)	18	5	95	1	3.2	96.8
Nalidixic acid (30 μg)	358	98.8	1.2	30	96.7	3.3
Enrofloxacin (5 μg)	261	72	28	23	74.1	25.9
Norfloxacin (10 μg)	239	66	34	21	67.7	32.3
Danofloxacin (5 μg)	246	67.9	32.1	20	64.5	35.5
Erythromycin (15μg)	353	97.5	2.5	30	96.7	3.3
Amoxicillin (20 μg)	355	98	2	29	93.5	6.5
Ampicillin (10 μg)	148	40.8	59.2	14	45.1	54.9
Tetracycline (10 μg)	210	58	42	13	41.9	58.1
Gentamycin (10 μg)	357	98.6	1.4	29	93.5	6.5

*n, number of susceptible *C. jejuni* and *C. coli* isolates, S, susceptible, R, resistant

Discussion

During the last decade, numerous studies have indicated *Campylobacter* infections as the primary bacterial infections of humans, and poultry products were discriminated as the main sources.

In this study, a high contamination rate of thermophilic *Campylobacter* was detected in faecal samples obtained from 2 broiler flocks, and carcasses, caecum, and carcass rinses from a local slaughterhouse. The prevalence of thermophilic *Campylobacter* spp. was determined as 91.8% in all samples examined. The isolation rate of *C. jejuni* (92.4%) was found higher than that of *C. coli* (7.6%).

Humphrey and Lanning (18) reported that 37 broiler flocks out of the 47 broiler flocks examined were positive (76%) for *C. jejuni*. Jacobs-Reitsma (19) indicated that faeces from 2 flocks were positive (100%) for thermophilic *campylobacter*s. Results of the present study are in agreement with those of previous studies.

In this study, thermophilic *Campylobacter* spp. was determined as 93.6% of carcass samples collected from a slaughterhouse. In a study, conducted by Yildiz and Diker (10) isolation rate of thermophilic *campylobacter*s from chicken carcasses was reported to be 100%. Smeltzer (20) showed that 94% of the carcasses were positive for thermophilic *campylobacter*s. Stern et al. (21), Shanker

et al. (22) and Lammerding et al. (23) found the carcass contamination rates of thermophilic *campylobacter*s as 21.3%, 45%, and 38.2%, respectively in 3 different slaughterhouses. Variations in the present isolation rates between these studies and ours can be due to several reasons, such as the difference of the local prevalence of *campylobacter*s in that specific region, and differences in the methods applied.

In several studies, Wempe et al (24), Baker et al. (25), Bryan and Doyle (26), Berrang et al. (1) and Buhr et al. (27) indicated that the rate of thermophilic *Campylobacter* contamination on chicken carcasses and their products was increased due to the possible faecal contamination throughout the processing line, particularly during and after evisceration in slaughterhouses. Wempe et al. (24) reported the average thermophilic *Campylobacter* contamination rate as 77% in two chicken processing plants in California, USA. Baker et al. (25) reported an increase in thermophilic *Campylobacter* contamination rates of the carcasses after evisceration in five chicken processing plants in New York State, USA. Similarly, Berrang et al. (1) and Buhr et al. (27) reported that thermophilic *Campylobacter* contamination of carcasses increased after the removal of intestinal organs. Bryan and Doyle (26) reported that the majority of raw poultry products at the processing and retail levels are contaminated with thermophilic *Campylobacter* spp. and are therefore a potential risk for humans in the USA. The high prevalence of *C. jejuni* in poultry processing plants, as shown in this study, ultimately results in contamination of the end-products.

All samples collected from cold water tank (51 °C) were found to be contaminated with thermophilic *campylobacter*s. In this study, there was no isolation from the water samples collected from the scald water tank (58 °C). These results indicated that the contamination of thermophilic *campylobacter*s might not originate from scald water tank. Humphrey and Lanning (18) reported that thermophilic *Campylobacter* spp. contamination was increased by evisceration of intestinal organs after defeathering. In their study, they showed that there was a cross-contamination between the cold water tank and the carcasses. Our results are in agreement with this study.

During the past decade, fluoroquinolones have been the principal agents in the prophlaxis and treatment of enteric infections. Unfortunately, there has been a rapid emergence of quinolone resistance amongst *Campylobacter* isolates all around the world (12,13).

Antibiotic susceptibility test results of this study indicate that there is an overall increase in the resistance of thermophilic *campylobacter*s to quinolones, amoxicillin, ampicillin, tetracycline, erythromycin and gentamycin. In the present study, a high frequency of resistance to the quinolone antibiotics was noted. Kramer et al. (5), Endtz et al. (12), Reina (13) and Gaudreau and Gilbert (14) previously reported an increase in resistance to fluoroquinolones in thermophilic *campylobacter*s, possibly as a result of discriminative use of these groups of antibiotics in veterinary practice.

In conclusion, the prevalence of thermophilic *campylobacter*s is high in both broiler farms and processing plants in Kirikkale, Turkey. Therefore, *Campylobacter* contamination of carcasses during processing constitutes a risk for consumers. All these results indicate that a preventive approach such as HACCP to eliminate the risk of *Campylobacter* contamination is required for both broiler farms and slaughterhouses.

Acknowledgements

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