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 carcasses, 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 *campylobacters* in broiler farms and slaughterhouses.

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

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**Broiler Tavuklarda Termofilik *Campylobacter* Türlerinin Prevalansı ve Antibiyotik Duyarlıkları**

**Özet:** Bu çalışmamın amacı, broiler karkas ve dışık örneklerinde termofilik *Campylobacter* türlerinin prevalansını araştırmaktır. Çalışmada, broiler kesimhanelerinde kesim sırası ve sonrasında karkasların dışık ve dolaysıyla *Campylobacter*ler ile olası kontaminasyon riski belirlendi. Ayrıca, bu çalışmada, broilerlerden izole edilen *Campylobacter* izolatlarının antibiyotik duyarlıklarını değerlendirildi.

Broiler 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. Broiler pillilerinden alınan rektal örneklerden %92,9 oranında izolasyon gerçekleşti. 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 karsılıklarından (%93,6) ve tavuk dışıklarından (%92,9) yapıldı. İzole edilen suçların %92,2’si *Campylobacter jejuni*, %7,8’i *C. coli* olarak identifiye edildi. *C. jejuni* izolatlarının %30,6’sı β-laktam antibiotiklerine ve %31,3’ü kinolon grubu antibiotiklere dirençli bulundu. *C. coli* izolatlarının ise %30,7’si b-laktam antibiotiklere ve %31,2’si ise kinolon grubu antibiotiklere dirençli bulundu.

Sonuçlar Kırıkkale broiler çiftliklerindeki broiler karsılıklarında termofilik *Campylobacter*ler ile yağın bir fekal kontaminasyonun olduğuunu ve bunun halk sağlığı açısından önemli bir risk oluşturduğunu göstermektedir. Bu çalışma, HACCP (Kritik Kontrol Noktalarında Tehlike Analizi) gibi koruyucu bir sistem uygulamasının *campylobacters*in broiler çiftlikleri ve kesimhanelerdeki kontaminasyon kontrolleri için gerekliliğini bir kez daha vurgulamaktadır.

**Anahtar Sözcükler:** Prevalans, antibiyotik duyarlılık, termofilik *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 Yardımı (8) reported that faeces from 7 flocks were positive for thermophilic campylobacters. 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.
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% O2, 6% CO2, 3% H2, and 85% N2) 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).
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 campylobacters. 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 campylobacters from chicken carcasses was reported to be 100%. Smeltzer (20) showed that 94% of the carcasses were positive for thermophilic campylobacters. Stern et al. (21), Shanker

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

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

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

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em>/362</td>
<td>S (%)</td>
</tr>
<tr>
<td>Cephalothin (30 µg)</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Nalidixic acid (30 µg)</td>
<td>358</td>
<td>98.8</td>
</tr>
<tr>
<td>Enrofloxacin (5 µg)</td>
<td>261</td>
<td>72</td>
</tr>
<tr>
<td>Norfloxacin (10 µg)</td>
<td>239</td>
<td>66</td>
</tr>
<tr>
<td>Danofloxacin (5 µg)</td>
<td>246</td>
<td>67.9</td>
</tr>
<tr>
<td>Erythromycin (15µg)</td>
<td>353</td>
<td>97.5</td>
</tr>
<tr>
<td>Amoxicillin (20 µg)</td>
<td>355</td>
<td>98</td>
</tr>
<tr>
<td>Ampicillin (10 µg)</td>
<td>148</td>
<td>40.8</td>
</tr>
<tr>
<td>Tetracycline (10 µg)</td>
<td>210</td>
<td>58</td>
</tr>
<tr>
<td>Gentamycin (10 µg)</td>
<td>357</td>
<td>98.6</td>
</tr>
</tbody>
</table>

* n, number of susceptible C. jejuni and C. coli isolates, S, susceptible, R, resistant
et al. (22) and Lammerding et al. (23) found the carcass contamination rates of thermophilic campylobacters 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 campylobacters 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 campylobacters. 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 campylobacters 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 prophylaxis 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 campylobacters 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 campylobacters, possibly as a result of discriminative use of these groups of antibiotics in veterinary practice.

In conclusion, the prevalence of thermophilic campylobacters 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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References


