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# The identification of *Campylobacter* contamination in chicken meat sold for human consumption in the Eşme district using nested PCR

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## Abstract

The Eşme district was chosen for this study based on food safety concerns in the area. *Campylobacter* spp. are the most prevalent bacteria responsible for food-borne bacterial diseases globally and are present in significant amounts in fowl gut flora. There are few effective methods for identifying *Campylobacter* in environmental samples, making it challenging to identify the cause of *Campylobacter* infections on chicken farms. Research on methods of identifying *Campylobacter* infections is therefore needed, particularly in areas where livestock husbandry is the main source of income. Due to the low bacterial concentration in samples and the possibility of uncultivable or fatally damaged bacterial stages, *Campylobacter* is difficult to identify in environmental samples using standard culture techniques. Furthermore, sensitivity is reduced because of the use of selective media. In this study, a nested polymerase chain reaction (PCR) method using hippuricase and 16S rRNA primers was employed to identify *Campylobacter jejuni* and *Campylobacter coli* in 55 chicken meat samples from the Eşme district. The sensitivity, specificity, and utility of PCR for detecting *C. jejuni* and *C. coli* in samples are examined.

**Keywords:** *Campylobacter coli*, food safety, *hipO* gene, real-time PCR, zoonotic disease

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## Introduction

In terms of cost, production efficiency, and health advantages, poultry meat is one of the most valuable sources of animal protein for humans (Daniel *et al.*, 2011). However, a number of factors, such as bacterial and parasitic infections, heat stress, and the ingestion of mycotoxins and oxidised lipids, can result in modern broiler production methods producing chickens with poor-quality meat and body composition (Choi *et al.*, 2023). Campylobacteriosis is one of the most prevalent bacterial intestinal diseases worldwide, with human infections primarily caused by two types of *Campylobacter*: *Campylobacter jejuni* or *Campylobacter coli* (Korsak *et al.*, 2015). According to Connerton *et al.* (2017), poultry and poultry products are significant contributors to human illness, with poultry acting as a major reservoir for the transfer of *Campylobacter* to humans (Poudel *et al.*, 2022).

Numerous potential sources of *Campylobacter* infections in broiler flocks have been identified through epidemiological investigations in the poultry industry, including water, insects, wild birds, rodents, and farmworkers (Stella *et al.*, 2017). Various measures have been implemented to prevent

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*Campylobacter* infections in broiler flocks, but these have had limited success. Improved hygienic barriers around broiler houses have been associated with a lower frequency of *Campylobacter* in broiler flocks, as shown in several European studies (Kuhn *et al.*, 2017). Strict cleanliness practices have been found to somewhat delay, but not fully prevent, the spread of *Campylobacter* to broiler flocks (Kuhn *et al.*, 2017). The precise origins of *Campylobacter* infections in poultry barns have not been identified in these studies.

Due to the low bacterial concentrations in collected samples, the use of selective media, and the potential presence of sublethally damaged stages, conventional culture methods for detecting *Campylobacter* in environmental samples have limited sensitivity (Vetchapitak *et al.*, 2019). As a result, identifying sources of *Campylobacter* infection and detecting *Campylobacter* in environmental samples requires the development of more sensitive and reliable approaches. Methods using polymerase chain reaction (PCR) technology offer a possible solution to these challenges.

Humans are commonly exposed to contaminated food items or water sources containing *C. jejuni*, a prevalent cause of gastroenteritis (Tikhomirova *et al.*, 2024). As a zoonotic disease, *C. jejuni* gastroenteritis is transmitted to humans from animals, primarily chickens, where it is a normal component of the intestinal microbiota. However, it can also be spread by cattle, pigs, sheep, and, as more recent research indicates, domestic cats and dogs (Thépault *et al.*, 2020). Humans often contract *C. jejuni* infections from contaminated milk, water, and infected animal food products, most frequently poultry products (Dessouky *et al.*, 2022). The hippuricase test (N-benzoylglycine amidohydrolase) is considered crucial for differentiating *C. jejuni* (hippuricase positive) from other *Campylobacter* species (hippuricase negative). Similarly, Totten *et al.* (1987) found that the key factor distinguishing *C. jejuni* from *C. coli* is the hippuricase (*hipO*) gene (Alarjani *et al*., 2021).

Numerous PCR assays have been effective in identifying *Campylobacter*, both directly from chicken faeces samples and from various other sources (Machiels *et al.*, 2000). These techniques also allow for the detection of both dead *Campylobacter* cells and non-culturable forms of the bacteria (Shane, 2000). According to Oberhelman & Taylor (2000), nested PCR assays can enhance sensitivity by using the PCR amplicon from the initial reaction as a template. In the secondary reaction, a corresponding set of internal primers is applied to the sequences amplified by the primary reaction. This study designed and used PCR methods with nested primer sets from 16S rRNA and *hipO* genes to identify *C. jejuni* and *C. coli* bacteria in chicken meat samples from retailers in the Eşme district.

## Material and Methods

Ethical approval and permission for this study was obtained from the Uşak University Animal Experiments Local Ethics Committee (date: 03/07/2024, decision no: 2024/01).

A nested PCR method was used to test for the presence of *C. jejuni* and *C. coli* bacteria in chicken meat cuts (leg, breast, and wing) that were packaged and sold in markets, delicatessens, and butchers in the Eşme district between February 2023 and March 2024. A total of 55 samples – 21 chicken wings, 16 chicken legs, and 18 chicken breast samples – were gathered between the designated dates and transported under cold chain conditions to the laboratory. Under laboratory conditions, 225 mL of Bolton Broth (Oxoid CM0983; without blood added), with Bolton Broth Selective Supplement (Oxoid SR0183E), was added to 25 g chicken meat samples in sterile stomacher bags. A homogeniser was used to blend the samples for 30–60 seconds. The enrichment bags' tops were rolled down to release any air, and the bags were then incubated for 24–48 hours at 41.5 ± 1 °C.

A portion of each sample was extracted from the enrichment bag and placed in a 2 mL tube after the incubation period. Zirconium beads, 10 µL of proteinase K, and 500 µL of extraction buffer were added to each 2 mL tube. The tissue sample was then homogenised until it disintegrated, and the tube was kept at 56 °C in a water bath for 15–20 minutes. A DNA isolation kit (Thermo Scientific™, K0722) was used to isolate the DNA from the samples, following the provided kit protocol. Extracted samples were then stored at either +4 °C or -20 °C for later use in the PCR process.

Polymerase chain reaction was performed using the primers listed in Table 1 to identify *Campylobacter* isolates. Polymerase chain reaction tubes provided with the PCR assay kit (Applied Biosystems™ MiniAmpTM, A37834) were warmed to room temperature (23 ± 5 °C), and the reagents mentioned in Table 2, apart from the DNA, were mixed and added to the wells. The PCR reaction was then initiated by adding the extracted DNA samples, and the prepared samples were incubated in a SimpliAmp™ Thermal Cycler using the operating program given in Table 3.

**Table 1** Primer sequences used for the polymerase chain reaction process

| Gene name | Primer sequences used |
|---|---|
| **16S rRNA (*Campylobacter* spp.)** | |
| CG12-F: | 5'-TTGATCCTGGCTCAGAGT-3' |
| CG1507-R: | 5'-TTCACCCCAGTCGCTGAT-3' |
| **16S rRNA (nested primer)** | |
| CcCj609-F: | 5'-ATCTAATGGCTTAACCATTA-3' |
| CcCj1442-R: | 5'-GTAACTAGTTTAGTATTCCGG-3' |
| ***HipO* (*Campylobacter jejuni*)** | |
| Hip100-F: | 5'-ACTGCAAAATTAGTGGCG-3' |
| Hip1128-R: | 5'-GAGCTTTTAGCAAACCTTCC-3' |

**Table 2** Volumes of reagents used for the polymerase chain reaction process

| PCR standard reaction (for one sample) | Volume (µL) |
|---|---|
| **HS master mix** | 10 |
| **Forward primer** | 0.7 |
| **Reverse primer** | 0.7 |
| **ddH$_2$O** | 6.3 |
| **DNA** | 3 |

**Table 3** Thermal cycler operating program for the polymerase chain reaction process

| Temperature (°C) | Time frame | Cycle | Stage |
|---|---|---|---|
| **95 °C** | 5 min | X1 | |
| **95 °C** | 15 s | | |
| **52 °C** | 30 s | X30 | Cycling stage |
| **72 °C** | 1 min | | |
| **72 °C** | 10 min | X1 | Post holding stage |
| **4 °C** | ∞ | X1 | |

Amplified PCR products were separated on 1% agarose gels using ethidium bromide at 100 V for roughly one hour. The DNA bands were then examined under ultraviolet light, and DNA ladder analysis was performed to determine the fragment sizes, specifically looking for the 16S rRNA and the 1148 bp fragment of the *hipO* gene. After sequencing of the positive isolates, the GenBank BLAST tool was used to confirm that the primer sequences were compatible with the target species.

## Results

To determine the presence of *Campylobacter* genus bacteria in the raw chicken meat samples (leg, breast, and wing meat), DNA was extracted, and PCR was performed using CG12-F and CG1507-R primers to amplify a 1495 bp product. The PCR results were visualised using a 2% agarose gel (Figure 1). All samples were found to be positive for *Campylobacter*.

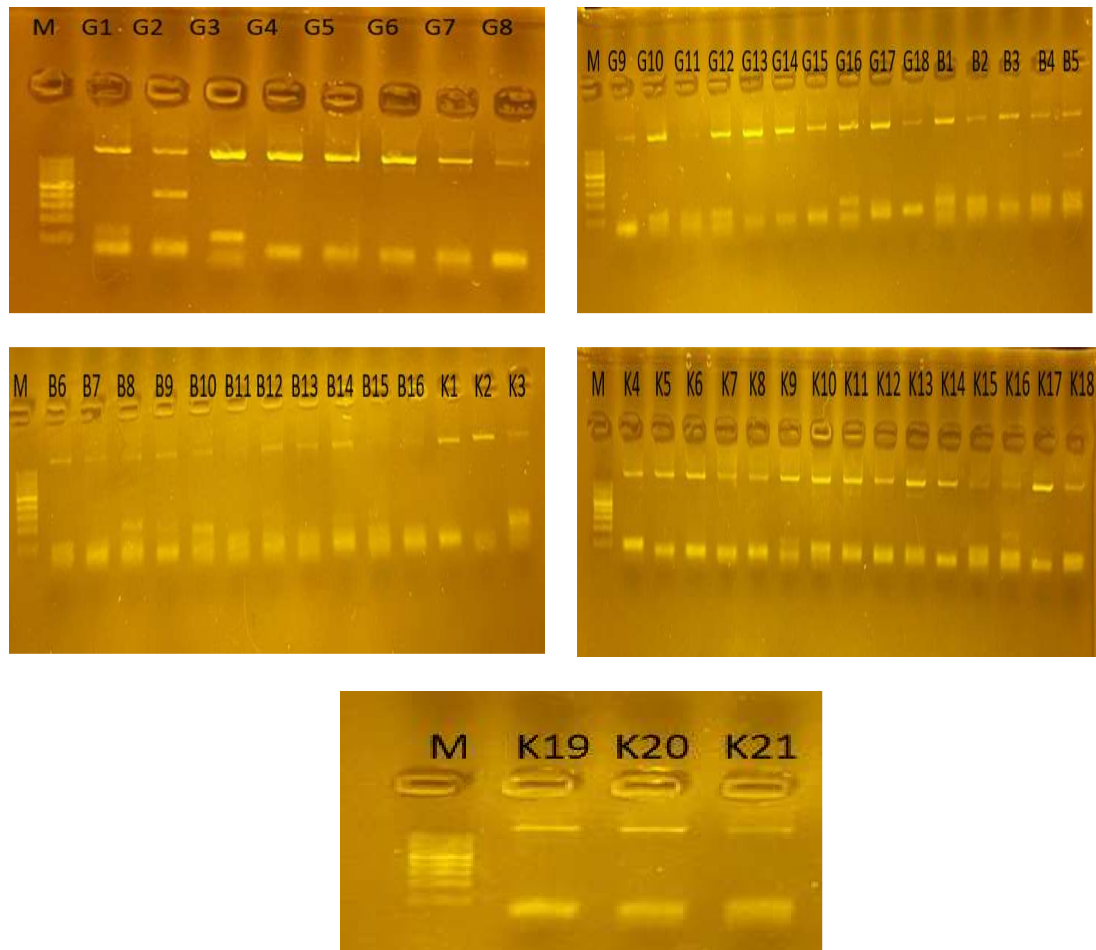


**Figure 1** Representative nested polymerase chain reaction for *Campylobacter* in 55 samples. M: molecular weight (1 Kb Plus DNA Ladder; Vivanti, catalogue number NL 1403); G: breasts; B: legs; K: wings

The 16S PCR products from the samples, along with CcCj609-F and CcCj1442-R primers, were used to identify bacteria within the *Campylobacter* family, including *C. jejuni* and *C. coli*. Samples that exhibited two bands on the 2% agarose gel were identified as containing *C. coli*, with a final product length of ca. 833 bp. The presence of single bands in other samples indicated the presence of several other *Campylobacter* species. The results of the 16S nested PCR agarose gel are shown in Figure 2.

To further identify *C. jejuni*, PCR amplification was performed using the Hip100-F and Hip1128-R primers on the 16S-positive samples. *C. jejuni* and *C. coli* were distinguished based on the *HipO* gene, as a unique portion of the *HipO* gene is specific to *C. jejuni*. The PCR results for the *HipO* gene revealed that no samples showed banding, indicating that *C. jejuni* was not detected in the targeted area (Figure 3).

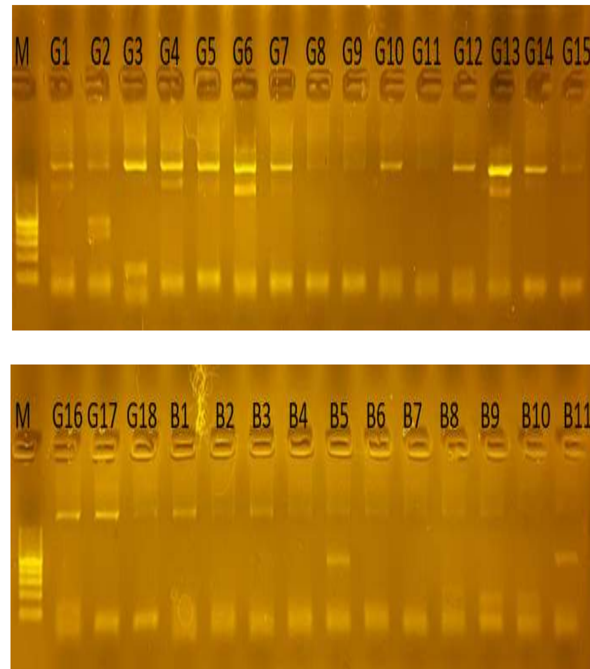


**Figure 2** Results of the 16S nested polymerase chain reaction agarose gel for *Campylobacter* family members (positive examples are G2, G4, G5, G6, G13, B5, B11). M: molecular weight, G: breasts, B: legs, K: wings

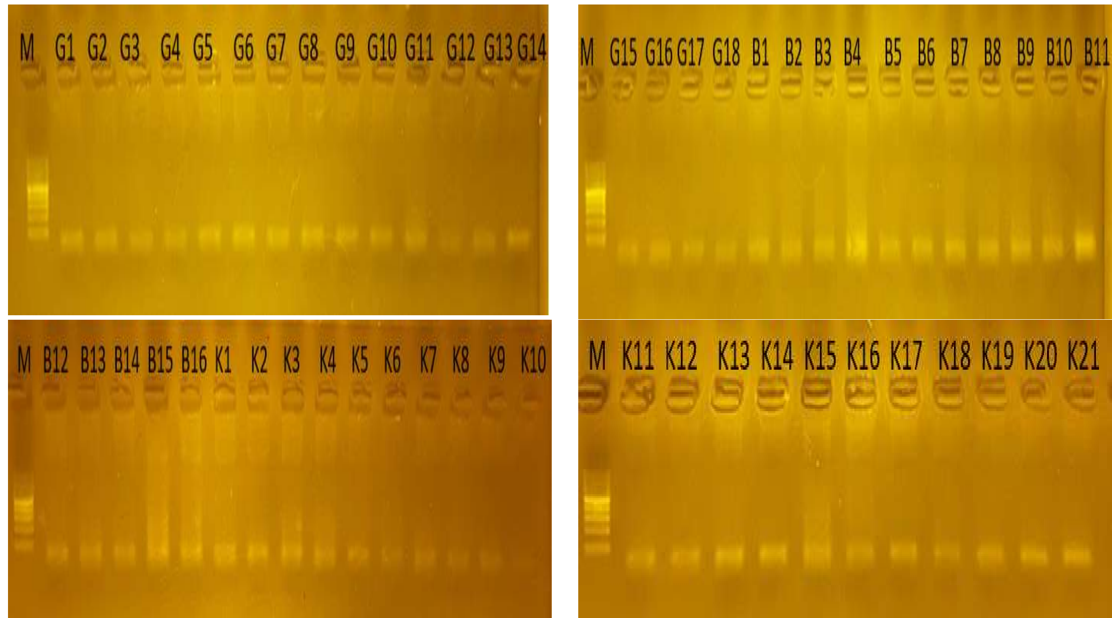


**Figure 3**. Results of the 16S nested polymerase chain reaction agarose gel for the identification of *Campylobacter jejuni* and *Campylobacter coli* (no band was observed in the 1028 bp region). M: molecular weight, G: breasts, B: legs, K: wings

Chicken meat samples from the breast (18), leg (16), and wing (21) were used for DNA isolation. The *Campylobacter* 16S region was amplified using CG12-F and CG1507-R primers, resulting in a final product of 1495 bp. Samples with bands at this size were considered positive for *Camplobacter*. The PCR products from these positive samples were then used as templates to differentiate *C. coli* and *C.*

*jejuni* from the *Campylobacter* family, using the primers CcCj609-F and CcCj1442-R. Band formation at 833 bp was observed in some of these samples, whole others, which did not form bands, were associated with different *Campylobacter* species. To detect *C. jejuni*, Hip100-F and Hip1128-R primers targeting the *HipO* gene were used, which also differentiated *C. jejuni* from *C. coli*. However, the *HipO* PCR revealed no *C. jejuni*-positive samples, as all tests were negative for the *HipO* gene.

The data acquired confirmed that the positive bands in the 16S nested PCR agarose gels corresponded to *C. coli* products, although several bands, other than 833 bp, were also detected. These additional bands were believed to be from *C. coli* substrains, indicating the presence of these substrains in the samples that tested positive for *C. coli*. The aim of this study was to determine whether *C. jejuni* and *C. coli* were present in chicken meat samples collected from various locations within the Uşak/Eşme district. The study concluded that multiple bacterial species within the *Campylobacter* family were present in the samples.

## Discussion

Of the 55 chicken pieces examined, 21.81% (12/55) were found to be contaminated with *Campylobacter* species (Table 4). Similar findings were reported by Di Giannatale *et al.* (2019), who tested 1243 poultry meat samples (665 chicken breasts and 578 chicken legs) from retail outlets and randomly selected supermarkets in various Italian regions. In their study, 131 samples tested positive or *Campylobacter*, with 57.96% being *C. jejuni* and 42.03% (95/228) being *C. coli*.

**Table 4** Presence of *Campylobacter* spp. in analysed chicken meat samples

| Sample | N | *Campylobacter* spp. | |
|---|---|---|---|
| | | n | % |
| Leg | 16 | 4 | 25 |
| Breast | 18 | 6 | 33.33 |
| Wing | 21 | 2 | 9.52 |
| Total | 55 | 12 | 21.81 |

N: total sample numbers, n: number of samples detected positive

There are notable distinctions between the contamination levels of different varieties of poultry meat sold in retail establishments. For example, when the skin has been removed, thighs have been found to be more contaminated than whole breasts or sliced meat (Ge *et al*, 2013). In this study, skinless breast meat had the highest contamination rate (6/18, 33.33%). This aligns with the results of Tedersoo *et al.* (2022), who detected *Campylobacter* spp. in 141 out of 429 chicken meat samples (32.9%). Poudel *et al.* (2022) found that in 414 chicken samples, the total prevalence of *Campylobacter* was 25.4% (105/414). However, Totten *et al.* (1987) suggested that hippurate hydrolysis should not be the only criterion employed for thermophilic *Campylobacter* differentiation, as they proposed that certain *C. jejuni* isolates were hippurate negative, even though *C. jejuni* is the only *Campylobacter* species possessing the *HipO* gene. Since our results were not consistently reproducible and weakly positive reactions can be interpreted in different ways, our study's findings support this approach (Engvall *et al.*, 2002).

Because of the paucity of baseline data, fresh chicken meat in retail and restaurant establishments is thought to have limited levels of *Campylobacter* contamination at the consumption stage (Kumagai *et al.*, 2020). However, Sasaki *et al.* (2011) found that 33% (198/600) of packaged chicken products produced by 22 broiler farms tested positive for *Campylobacter*. Furthermore, Mwacharo *et al.* (2011) found that 80% of skin samples taken from carcasses after cooling and 100% of skin samples after evisceration tested positive for *Campylobacter*.

*Campylobacter* infections constitute a major zoonotic food-borne disease, prevalent worldwide, and pose a significant hazard to public health. As *C. jejuni* is a normal component of the intestinal flora of chickens, contamination frequently occurs at slaughterhouses when hygienic procedures are not followed. The results of this study differ from those of previous studies, possibly due to factors such as sample size, the time of year the samples were collected, and the analytical techniques employed.

Furthermore, the prevalence of contamination is influenced by the degree of adherence to hygienic standards throughout the entire production process in the areas where the samples were collected, as well as the producers' awareness of their responsibility and the regularity of inspections. More efforts should therefore be made to inform the public about the health risks associated with the preparation and consumption of chicken meat, as well as to reduce the level of *Campylobacter* contamination in chicken meat. Adopting multidisciplinary techniques can lead to improvements, as demonstrated by previous cases in various countries (Sears *et al.*, 2011; Tustin *et al.*, 2011).

## Conclusion

In this study, 21.81% of the chicken samples tested were found to be contaminated with *Campylobacter*, with the contamination rate varying across different cuts. Breast meat exhibited the highest prevalence of contamination. This high prevalence of *Campylobacter* contamination in retail chicken meat is a persistent concern for public health.

The occurrence of *Campylobacter* can be influenced by several factors, including cross-contamination, insufficient heat treatment, live animal infection, and mistakes made during product transit and storage. It is therefore critical to prevent the disease from spreading between farms, promote intensive farming practices, adhere to biosecurity procedures, and apply pesticides and rodenticides as needed. Slaughterhouses must exercise exceptional caution during the slaughtering process, taking extra care to prevent contamination during the removal of internal organs and feathers. During storage, portioned meats and chicken carcasses should be frozen or refrigerated. Consumers should also take precautions to avoid cross-contamination when preparing and consuming meat and chicken products. Hygiene should be prioritised at every stage of production, including cleaning, sanitation, personnel, air, water, and equipment. Additionally, internal controls and regular audits must be conducted.

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### Conflicts of interest

The author declares no conflicts of interest for this article.

### Availability of data and material

Data will be available on request.

### Authors' contributions

Esra Bilici – planning, conception, design, analysis, interpretation, writing a draft of the article, final approval of the version to be published. Author reviewed and approved the manuscript before it was submitted for publication.

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