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Original Scientific Paper

Occurrence and species-level identification of thermophilic Campylobacter in retail poultry meat in Serbia

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ABSTRACT

Species within the *Campylobacter* genus, notably *Campylobacter jejuni* and *Campylobacter coli*, are among the primary bacterial agents responsible for foodborne illnesses worldwide. Poultry meat frequently serves as a key vector of transmission, owing to the high prevalence of these bacteria in the gastrointestinal tracts of birds. The purpose of this study was to assess both the occurrence and levels of *C. jejuni* and *C. coli* in raw chicken meat available in Serbian retail markets. In 2023, a total of 118 fresh poultry samples were obtained from various retail outlets and analyzed in accordance with ISO standards for qualitative and quantitative microbiological evaluation. Results indicated a notably high contamination rate, with *Campylobacter* detected in 75% of the samples. Among these, 63 samples exhibited quantifiable bacterial loads ranging from 20 to 7,600 CFU/g. Molecular testing further confirmed the presence of thermotolerant *Campylobacter* species in 98% of the positive samples. Specifically, *C. jejuni* was detected in 75%, and *C. coli* in 85% of cases, with over half of the positive samples containing both species. The highest bacterial concentrations were found in drumstick and thigh meat cuts. These results emphasize the considerable public health concern associated with *Campylobacter* contamination in poultry meat sold in Serbia, and they reinforce the necessity of stricter hygiene practices and comprehensive monitoring across the poultry production and distribution chain.

1. Introduction

Species of the genus *Campylobacter*, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as leading contributors to bacterial gastroenteritis transmitted through food on a global scale. Estimates suggest that approximately 96 million infections occur annually as a result of these pathogens (*Majowicz et al.,* 2020). These thermophilic, Gram-negative organisms are commonly found in the intestinal tracts of poultry, where they typically do not induce symptoms in the host. However, during slaughtering and processing, they can be transferred from the intestines to the surface of poultry carcasses, creating a significant potential for contamination of meat destined for retail markets (*Sahin et al*., 2015; *Lopes et al.,* 2021).

Human exposure is frequently linked to the consumption of raw or undercooked poultry products, as well as to inadequate handling practices during food preparation (*Lopes et al.,* 2021). Due to the low infectious dose—often fewer than 500

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viable cells—even minimal levels of *Campylobacter* in meat can pose a notable threat to public health (*Black et al.,* 1988).

Although *Campylobacter* has been consistently reported as the most prevalent foodborne pathogen in the European Union since 2005 (*EFSA,* 2023), data from non-EU countries such as Serbia remain sparse. Serbia introduced surveillance of campylobacteriosis in humans in 1997 and implemented mandatory testing of poultry carcasses in slaughterhouses starting in 2019. Nevertheless, only a limited number of studies have explored the presence of *Campylobacter* spp. specifically in chicken meat at the retail level—an important point of consumer exposure.

Reliable baseline data on the occurrence and levels of *Campylobacter* in chicken meat sold through retail channels are crucial for evaluating consumer risk and informing appropriate control strategies (*Stella et al.,* 2017). Unlike farm-level testing, retail surveillance reflects the cumulative impact of contamination throughout the entire poultry production and distribution process. Furthermore, since *Campylobacter* does not multiply under refrigeration, quantification at the point of sale offers a realistic estimate of the microbial load to which consumers may be exposed (*Habib et al.,* 2022).

Reports from the EU demonstrate substantial variability in the prevalence of *Campylobacter* in retail poultry—ranging from 1.8% in Estonia to nearly 100% in Italy—indicating the need for country-specific investigations (*Wieczorek et al.,* 2012; *Belkacem et al.,* 2023). In Serbia, the incidence of human campylobacteriosis was reported at 4.04 cases per 100,000 inhabitants in 2022 (*IPHS,* 2023), yet the degree of exposure through retail poultry products remains insufficiently documented. While prior research has identified contamination levels as high as 85% in broiler carcasses at the slaughter stage (*Jovanović et al., 2020*), comprehensive data from the retail sector are lacking.

In light of these gaps, the present study aims to assess both the prevalence and levels of *C. jejuni* and *C. coli* in raw chicken meat obtained from retail stores across Serbia. The findings are expected to contribute to a deeper understanding of poultry meat safety and support the development of targeted interventions to mitigate public health risks associated with *Campylobacter* contamination.

2. Materials and methods

2.1. Sample collection

In 2023, a total of 118 fresh poultry meat samples were aseptically collected from retail outlets across Serbia. Sample types were drumsticks, thighs, fillets, wings, backs, breasts, leg quarters, whole grill chickens, and schnitzels. All samples were transported to the laboratory under controlled temperature conditions for microbiological analysis.

2.2. Detection and enumeration of Campylobacter spp.

Isolation and quantification of *Campylobacter* spp. were performed according to SRPS EN ISO 10272-1:2017/A1:2023 (qualitative; *ISO*, 2023a) and SRPS EN ISO 10272-2:2017/A1:2023 (quantitative; *ISO*, 2023b), with a detection limit of 10 CFU/g.

2.2.1. Qualitative detection

For enrichment, 25 g of sample was homogenized in 225 mL of Bolton broth and incubated under microaerophilic conditions: 4 h at 37 ± 1°C followed by 44 h at 41.5 ± 1°C. A 10 µL aliquot from the broth was streaked onto CASA agar (*bioMérieux, France*) and incubated at 41.5 ± 1°C for 48 h. Presumptive colonies were confirmed as per standard protocol.

2.2.2. Quantitative detection

For enumeration, 25 g of meat was homogenized in 225 mL of buffered peptone water. Aliquots (3×0.33 mL and 0.1 mL) were plated on CASA agar, including decimal dilutions. Plates were incubated at 41.5 ± 1°C for 48 h under microaerophilic conditions. Colony counts and identification followed the standard procedures.

2.3. Molecular confirmation of Campylobacter spp.

DNA was extracted from 91 samples, including 88 poultry isolates and 3 reference strains (*C. jejuni* ATCC 33291, *C. coli* ATCC 43478, *C. lari* ATCC 35223). The extraction involved lysis with buffer and Proteinase K, followed by isopropanol precipitation, ethanol washing, and resuspension in DNA storage buffer. DNA concentration and purity (A260/A280) were measured by spectrophotometry and standardized to 100 µg/mL.

2.3.1. Genus-level identification (Real-Time PCR)

Genus-level identification was conducted by targeting the 16S rRNA gene using species-conserved primers and a fluorogenic probe. PCR was performed using the Aria MX system (*Agilent Technologies*) and Brilliant III Ultra-Fast QPCR Master Mix (*Agilent Technologies*), following thermal cycling conditions from SRPS EN ISO 10272-1:2017/A1:2023, Annex D.

2.3.2. Species-level confirmation

Species-specific Real-Time PCR assays were used to identify *C. jejuni*, *C. coli*, and *C. lari* using primers and probes targeting mapA, ceuE, and gyrA genes respectively, according to Annex E of the ISO standard.

3. Results

A total of 118 fresh poultry meat samples were analyzed during the study. Of these, 88 samples tested positive for *Campylobacter* spp., corresponding to an overall prevalence of 75%. The distribution of positive samples across different poultry meat categories is presented in Table 1.

Enumeration of *Campylobacter* spp. using ISO 10272-2 (*ISO*, 2023b) revealed quantifiable contamination in 63 positive samples, with bacterial counts ranging from 20 to 7600 CFU/g. The highest contamination levels were observed in the drumstick and thigh category, ranging from 20 to 7600 CFU/g, whereas no colonies were detected in the chicken schnitzel group. Notably, the chicken back category showed a high contamination level of 4200 CFU/g despite the small number of samples. Qualitative detection of *Campylobacter* spp. using ISO 10272-1 (*ISO*, 2023a) revealed an additional 25 positive samples with contamination levels below 10 CFU/g.

Molecular analysis confirmed the presence of thermotolerant *Campylobacter* species in 98% of positive samples. Species-level identification revealed that *C. jejuni* was present in 75% of samples, while *C. coli* was identified in 85% of samples. *Campylobacter lari* was not detected in any chicken sample. Co-occurrence of *C. jejuni* and *C. coli* was found in 58% of samples. Additionally, *C. coli* was identified as a single species in 27% of samples, and *C. jejuni* alone was found in 15% of samples (Figure 1).

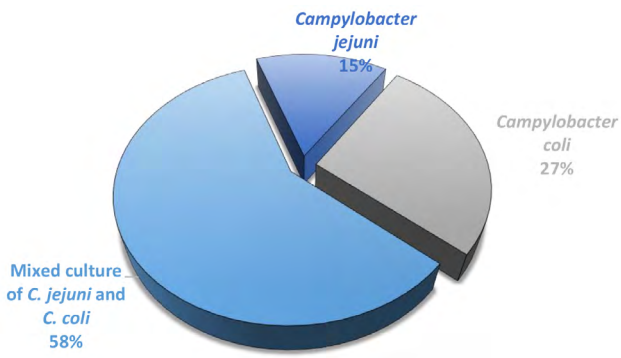

Figure 1. Species identification of *Campylobacter* isolates

Table 1. Prevalence of Campylobacter spp. in poultry meat by product category

Sample category	No. of samples	Negative samples	Positive samples	Prevalence (%)
Chicken drumstick	14	3	11	79%
Drumstick and thigh	30	4	26	87%
Chicken fillet	30	13	17	57%
Chicken wings	11	0	11	100%
Chicken back	2	1	1	50%
Chicken breast	10	3	7	70%
Chicken hindquarter	3	2	1	33%
Whole grill chicken	6	0	6	100%
Chicken thigh	8	2	6	75%
Chicken schnitzel	4	2	2	50%
Total	**118**	**30**	**88**	**75%**

4. Discussion

The findings of this study indicate a considerably high prevalence of *Campylobacter* spp. in retail poultry meat in Serbia, with 75% of the analyzed samples testing positive. Comparable results have been reported in nearby countries. For example, research in Croatia found a prevalence rate of 73.86% in chicken meat sold at retail level (*Mikulić et al.,* 2016). These regional similarities could suggest that common agricultural practices, meat processing systems, and conditions in the retail chain collectively contribute to the widespread contamination with *Campylobacter*. At the European level, *Campylobacter* has consistently been recognized as one of the most frequently encountered foodborne pathogens. According to the European Food Safety Authority's (EFSA) One Health Zoonoses Report for 2023, this bacterium was the most commonly detected zoonotic agent in the EU, particularly in broiler chicken carcasses, with a prevalence of 12% and in live broilers, with the prevalence of 18.1% (*EFSA,* 2023). These statistics reflect the need for rigorous and ongoing monitoring across poultry supply chains throughout Europe. Prevalence rates differ between countries. For instance, a study from France reported a 76% contamination rate in broiler meat products (*Guyard-Nicodème et al.,* 2015), and findings from Greece indicated a prevalence as high as 90% in chicken products sold at retail (*Kostoglou et al.,* 2023). In Poland, a study found a prevalence of 70% in chicken meat samples (*Szosland-Fałtyn et al.,* 2018). In contrast, some countries reported lower rates, such as 50% in Spain (*Perez-Arnedo & Gonzalez-Fandos,* 2019) and 60% in the United Kingdom (*FSA,* 2018). These variations could arise due to differences in surveillance approaches, local farming systems, or sampling strategies.

Within this study, prevalence also differed by product type. Chicken wings and whole grilled chickens were the most frequently contaminated (100%), while chicken schnitzels had the lowest rate (50%). A study conducted in Nova Scotia, Canada, found that chicken thighs had the highest prevalence of *Campylobacter* contamination among various retail poultry cuts, with 73.9% of thigh samples testing positive. In contrast, chicken wings had a lower prevalence of 58% (*Hodges et al.,* 2019). Similarly, research in Italy reported that chicken thighs had a higher occurrence of *Campylobacter* compared to other cuts, including breasts and wings (*Mezher et al.,* 2016). That study highlighted the anatomical proximity of thighs to the digestive tract as a potential factor influencing higher contamination rates (*Mezher et al.,* 2016). Factors such as anatomical location, handling practices, and storage conditions likely influence contamination levels.

Quantitative analysis of the samples in this study revealed bacterial loads ranging from 20 to 7,600 CFU/g, indicating considerable variability. *Campylobacter* spp. loads were also measured in Estonian broiler chicken products. Enumeration data reported by *Mäesaar et al.* (2014) indicated an overall arithmetic mean of 1.6×10^3 CFU/g of product. In contrast, the quantitative results of the study published by *Szosland-Fałtyn et al.*, (2018), demonstrated a low level of *Campylobacter* spp. contamination in the examined poultry meat samples. Specifically, *Campylobacter* spp. counts were below 10 CFU/g in 68% of the positive samples. Additionally, 22% and 26% of the samples exhibited pathogen concentrations ranging from ≥10 to <100 CFU/g. These results clearly shown needing for improved hygiene measures across all stages of production and distribution to reduce microbial risks.

Molecular identification confirmed *C. jejuni* and *C. coli* as the dominant species in the current study, present in 75% and 85% of positive samples, respectively. In Croatia, *C. jejuni* and *C. coli* were isolated from 53.53 and 15.35% of the samples, respectively (*Mikulić et al.,* 2016). In North Macedonia, *C. jejuni* was found in 39.2 of examined samples from broiler meat production (*Angelovski et al., 2021*). These findings demonstrate the widespread distribution of these pathogens in poultry meat across the continent.

5. Conclusion

The high prevalence and differing contamination levels of *Campylobacter* spp. in retail poultry meat in Serbia pose significant public health risks, as these bacteria are major causes of foodborne gastroenteritis worldwide. The European Food Safety Authority (EFSA) has emphasized the need for continuous monitoring and control of *Campylobacter* in the food chain to protect public health (*EFSA,* 2023). Implementing stringent hygiene practices in poultry processing and retail environments, along with consumer education on proper meat handling and cooking, are essential steps in mitigating the risks associated with *Campylobacter* contamination.

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