

Original Article

Isolation and characterization of thermophilic *Campylobacter* species from geese raised in Kars region (Turkey) using cultural, molecular and mass spectrometry methods

Demiroğlu, E. G.¹; Şahin, M.^{2*} and Büyük, F.²

¹Ph.D. Student in Microbiology, Department of Microbiology, Institute of Health Sciences, Kafkas University, Kars, 36100, Turkey;

²Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, Kars, 36100, Turkey

*Correspondence: M. Şahin, Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, Kars, 36100, Turkey. E-mail: mitats@hotmail.com

 10.22099/IJVR.2021.41103.5962

(Received 5 Jul 2021; revised version 8 Nov 2021; accepted 24 Nov 2021)

Abstract

Background: Thermophilic *Campylobacters* are found in the digestive tract of wild and domestic poultry and can be transmitted to humans following their fecal discharges. **Aims:** This study aimed to isolate thermophilic *Campylobacter* by culture from cloacal swabs of geese, commonly breeding in Kars region, and to identify the isolates by PCR and mass spectrometry. Antibiotic susceptibility and resistance genes of the isolates were also analysed. **Methods:** The study included 400 cloacal swab samples of clinically healthy geese. The samples were cultured on mCCDA medium following the pre-enrichment in Preston broth. Identification of the isolates was performed by phenotypic methods, PCR, and MALDI-TOF MS. Antibiotic susceptibility and resistance genes of the isolates were analysed with the disc diffusion method and PCR, respectively. **Results:** Thermophilic *Campylobacter* spp. were isolated from 157 (39.3%) samples. 151 (96.2%) isolates were identified *Campylobacter jejuni* and 6 (3.8%) *Campylobacter coli* by the phenotypic tests and PCR. Among 125 isolates analysed by MALDI-TOF MS, 119 (95.2%) were identified *C. jejuni* and 6 (4.8%) *C. coli*. The isolates' resistance to ampicillin, tetracycline, ciprofloxacin, gentamicin, and azithromycin were found 33.8%, 41.4%, 75.2%, 12.1%, and 7.6%, respectively. The distributions of *bla_{OXA61}*, *tetO*, *gyrA*, and *aphA-3* genes were 3.2%, 90.8%, 50.8%, and 52.7%, respectively. **Conclusion:** Since geese are raised in pastures in the Kars region, protecting and not polluting the existing natural environment and preventing their contact with wild birds will prevent the spread of these microorganisms.

Key words: Antibiotic susceptibility, Goose, MALDI-TOF MS, PCR, Thermophilic *Campylobacter*

Introduction

In Turkey, goose breeding is intensively carried out in Kars and its surroundings. It has traditional consumption habits, especially dried goose meat. Geese have some known bacterial and viral diseases. It is important to determine the presence and frequency of *Campylobacter*, an important bacterial species in terms of human and animal health and carried by poultry in the digestive system. *Campylobacter* species are important in transmission to domestic and wild animals and humans by contaminating environments through their primary hosts (Jones, 2011). The transmission to humans usually occurs by the oral route through contact with contaminated poultry products. Cloacal colonization, leading to contamination of poultry meat, is an important factor in the epidemiology and transmission of the thermophilic species (Coker *et al.*, 2002).

Cloacal colonization of the thermophilic *Campylobacter* may vary with age and seasonality (Ringoir *et al.*, 2002). In poultry, contamination and first colonization following fecal scattering begin at 2-3

weeks of age, and the spread of the microorganism to the flock increases with age (Evans and Sayers, 2000). The bacterial transmission among animals after the initial infection is extremely fast, and most of the animals in the herd are colonized in just a few days (Shreeve *et al.*, 2000). While colonization continues for life in poultry, the persistence of colonization may vary among the *Campylobacter* species (Ringoir *et al.*, 2002). It has also been reported that seasons are also effective in cloacal colonization (Refregier-Petton *et al.*, 2001). Generally, a higher prevalence was reported in summer months when environmental exposure is increased (Evans, 1997).

Isolation of the bacteria using cultural methods with mCCDA, Columbia, Skirrow, and Butzler agar is considered the gold standard in the diagnosis of thermophilic *Campylobacter*. Morphological, cultural, and biochemical tests are widely used to characterize the bacteria. However, alternative diagnostic methods are required due to the difficulties in the cultures, time-consuming process, or the morphological variations of *Campylobacter* in subcultures (live but non-culturable forms-VBNC). In this scope, PCR, which provides

enzymatic amplification of the bacteria-specific genes, and MALDI-TOF MS, which provides protein profiles following the ionization of bacterial biomolecules and organic molecules, provide faster and more reliable results (Seng *et al.*, 2009).

No antibiotic treatment is usually required for *Campylobacter* infections in poultry, while it may be necessary in humans, especially in cases with severe symptoms or immunodeficiency. Macrolides such as erythromycin and azithromycin are the most commonly used antimicrobials. Fluoroquinolones, tetracyclines, and aminoglycosides are other alternative medicines (Hasdemir, 2007). However, as a result of the unconscious and widespread use of antibiotics as prophylactic and growth factors especially in poultry breeding, antibiotic resistance in thermophilic species becomes more common and even multi-drug resistances have emerged (Wieczorek and Osek, 2013). These resistances are mainly based-on the presence of some genes such as *aphA-1*, *aphA-3*, *aacA*, *aphD*, and *aac* for aminoglycosides (Lambert *et al.*, 1985), *tetO* for tetracyclines (Chopra and Roberts, 2001), *gyrA* for fluoroquinolones (Luangtongkum *et al.*, 2009), *ermB* for macrolides (Qin *et al.*, 2014), and *bla_{OXA-61}* for β -lactams (Komba *et al.*, 2015).

This study aimed to isolate thermophilic *Campylobacter* from cloacal swab samples from geese of different age groups, which are widely raised in the Kars region, and to identify the isolates with molecular and MALDI-TOF MS. Another goal of the study is to determine the susceptibility of isolates to various antibiotics and to analyze the common resistance genes by PCR.

Materials and Methods

Study material

Ethical permission of the study was approved by the decision of Kafkas University Animal Experiments Local Ethics Committee, with the number "2019-124".

In this study, 400 cloacal swab samples were taken from clinically healthy geese from family-type farms in the center and districts of Kars (Turkey) between January and October 2020. In order to evaluate the seasonal distribution of thermophilic *Campylobacter*, sampling was done every month of the year with 40 cloacal samples.

Isolation and identification of thermophilic *Campylobacter*

The cloacal swab samples were pre-enriched in 5 ml *Brucella* broth with the addition of *Campylobacter* growth supplement and Preston *Campylobacter* selective supplement under microaerobic conditions at 42°C for 48-72 h (Anonymous 1, 2020). Then, 20 μ L pre-enriched content was inoculated onto mCCDA selective medium supplemented with *Campylobacter* Selective Supplement; the seeded agar plates were incubated under the same conditions. Following the incubation, colonies with 0.5 mm diameter, grayish color, sometimes metallic highlights, smooth surface but tendency to spread were evaluated as suspicious for thermophilic *Campylobacter* on mCCDA medium. Within the scope of preliminary identification of thermophilic *Campylobacter*, their microscopic morphology (Gram-negative, curved or comma-shaped bacillus), biochemical activities (oxidase and catalase activities, hippurate hydrolysis and indoxyl acetate reaction), and susceptibility to various antibiotics (nalidic acid and cephalothin) were evaluated (Anonymous 1, 2020).

Molecular identification

DNA extraction from suspected colonies was performed with a single colony lysis buffer solution (SCLB) supplemented with heat treatment (Mamur, 1961).

For the molecular identification of the thermophilic *Campylobacter* species, a multiplex polymerase chain reaction (mPCR) method was applied (Wang *et al.*, 2002).

For this purpose, total mPCR volume for each sample was adjusted as 25 μ L with the components of 2.5 μ L template DNA, 2.5 μ L 10x PCR buffer, 3 μ L MgCl₂ (20 mM), 0.5 μ L dNTP mix (0.2 mM), 1 μ L of each primer (20 pmol) and 0.5 μ L Taq polymerase (1.25 U). Thermal cycle of the mPCR was carried out by initial denaturation at 95°C for 6 min, followed by 30 cycles, with denaturation at 95°C for 30 s, primer attachment at 59°C for 30 s and elongation at 72°C for 30 s, and finally elongation at 72°C for 7 min. The amplified products were analyzed by 1.5% agarose gel electrophoresis, and products of 323 bp for *C. jejuni*, 126 bp for *C. coli*, 251 bp for *C. lari* and 204 bp for *C. upsaliensis* were evaluated (Table 1).

Table 1: Primer sequences used for mPCR

| Target gene | Primer sequence (5'-3') | Band size | Reference |
|---------------------------------------|--|-----------|---------------------------|
| <i>C. jejuni</i> (<i>hipO</i>) | ACTTCTTTATTGCTTGCTGC GCCACAACAAGTAAAGAAGC | 323 bp | Wang <i>et al.</i> (2002) |
| <i>C. coli</i> (<i>glyA</i>) | GTAAAACCAAAGCTTATCGTG TCCAGCAATGTGTGCAATG | 126 bp | Wang <i>et al.</i> (2002) |
| <i>C. lari</i> (<i>glyA</i>) | TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC | 251 bp | Wang <i>et al.</i> (2002) |
| <i>C. upsaliensis</i> (<i>glyA</i>) | AATTGAACTCTTGCTATCC TCATACATTTTACCCGAGCT | 204 bp | Wang <i>et al.</i> (2002) |

Identification with mass spectrometry

Identification of the thermophilic *Campylobacter* species by MALDI-TOF MS (Vitek MS, Biomeriux) was performed in Malatya İnönü University (Turkey). The isolates were subjected to the routine culture processes in proper media, and a fresh colony was taken with a loop to place on the MALDI TOF plate. Then, 1 µL α-cyano-4-hydroxynamic acid (α-CHCA) matrix solution was added to the well and dried. Identification was done by leaving the plate in the MALDI-TOF MS device for analysis (Yakupoğulları *et al.*, 2019).

Determination of antibiotic susceptibility of the isolates

The susceptibility of the identified thermophilic *Campylobacter* species against azithromycin (15 µg), tetracycline (30 µg), ciprofloxacin (15 µg), gentamicin (10 µg), and ampicillin (10 µg) (OXOID) was determined by Kirby-Bauer disc diffusion method (Taremi *et al.*, 2006). The inhibition zone diameters formed at the end of the test were measured and evaluated according to the CLSI 2017 criteria (CLSI, 2017). During the test, *Escherichia coli* ATCC® 25922, *Escherichia coli* ATCC® 35218, and *Pseudomonas aeruginosa* ATCC® 27853 were used as control bacteria.

Resistance gene analysis of the isolates

Of the phenotypically resistant thermophilic *Campylobacter* isolates, the resistance genes of

tetracycline (*tetO*), gentamicin (*aphA-3*), ampicillin (*blaOXA-61*), azithromycin (*ermB*), and ciprofloxacin (*gyrA*) were analysed by PCR (Table 2).

Statistical analysis

SPSS (Statistical Package for Social Sciences for Windows) 20.0 package software was used for statistical analysis. Mann-Whitney test was used for statistical comparison of the proportional distribution between two data values. The Kruskal-Wallis test was also employed for statistical comparisons of the proportional distribution between data of three or more experiments.

Results

Isolation and identification

As a result of cultural analysis, thermophilic *Campylobacter* isolation was performed in 157 (39.3%) of 400 goose cloacal swab samples. Out of 157 thermophilic *Campylobacter* isolates, 151 (96.2%) were identified as *C. jejuni* and 6 (3.8%) as *C. coli*. *C. lari* and *C. upsaliensis* were not detected in the study. Considering the cloacal colonization of thermophilic species by seasons, it was determined that the isolation rate started to increase in the spring, reached a peak in the summer, and gradually decreased in the autumn and winter ($P < 0.05$) (Table 3).

In order to test the age-related colonization of thermophilic *Campylobacter*, ten 1-day-old goslings

Table 2: Primer sequences used for PCR to analyze resistance gene

| Target gene | Primer sequence (5'-3') | PCR reaction | | |
|------------------|---|----------------|-----------|----------------------------|
| | | Annealing (°C) | Band size | Reference |
| <i>tet(O)</i> | GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC | 51 | 559 bp | Pratt and Korolik (2005) |
| <i>aphA-3</i> | TGCGTAAAAGATACGGAAG CAATCAGGCTTGATCCCC | 52 | 701 bp | Obeng <i>et al.</i> (2012) |
| <i>blaOXA-61</i> | AGAGTATAATACAAGCG TAGTGAGTTGTCAAGCC | 46 | 372 bp | Obeng <i>et al.</i> (2012) |
| <i>ermB</i> | CAGGTAAAGGGCATTAAACGACG CATCTGTGGTATGGCGGGTAAG | 60 | 738 bp | Zhou <i>et al.</i> (2016) |
| <i>gyrA</i> | GCTCTTGTTTTAGCTTGATGC TTGTCGCCATCCTACAGCTA | 56 | 620 bp | Wang <i>et al.</i> (1993) |

Table 3: Sample characteristics and test results used in identification

| Month | Sample size | Isolation rate | PCR | MALDI-TOF MS | Percentage (%) | Mann-Whitney P-value |
|-----------|-------------|----------------|---|---|----------------|----------------------|
| January | 40 | 11 | 10 <i>C. jejuni</i> , 1 <i>C. coli</i> | 9 <i>C. jejuni</i> , 1 <i>C. coli</i> | 27.5 | P<0.05 |
| February | 40 | 13 | 12 <i>C. jejuni</i> , 1 <i>C. coli</i> | 8 <i>C. jejuni</i> , 1 <i>C. coli</i> | 32.5 | |
| March | 40 | 12 | 12 <i>C. jejuni</i> , - | 8 <i>C. jejuni</i> , - | 30 | |
| April | 40 | 15 | 14 <i>C. jejuni</i> , 1 <i>C. coli</i> | 11 <i>C. jejuni</i> , 1 <i>C. coli</i> | 37.5 | |
| May | 40 | 20 | 19 <i>C. jejuni</i> , 1 <i>C. coli</i> | 17 <i>C. jejuni</i> , 1 <i>C. coli</i> | 50 | |
| June | 40 | 21 | 20 <i>C. jejuni</i> , 1 <i>C. coli</i> | 17 <i>C. jejuni</i> , 1 <i>C. coli</i> | 52.5 | |
| July | 40 | 26 | 25 <i>C. jejuni</i> , 1 <i>C. coli</i> | 19 <i>C. jejuni</i> , 1 <i>C. coli</i> | 65 | |
| August | 40 | 23 | 23 <i>C. jejuni</i> , - | 18 <i>C. jejuni</i> , - | 57.5 | |
| September | 40 | 10 | 10 <i>C. jejuni</i> , - | 6 <i>C. jejuni</i> , - | 25 | |
| October | 40 | 6 | 6 <i>C. jejuni</i> , - | 6 <i>C. jejuni</i> , - | 15 | |
| Total | 400 | 157 | 151 <i>C. jejuni</i> , 6 <i>C. coli</i> | 119 <i>C. jejuni</i> , 6 <i>C. coli</i> | 39.3 | |

were sampled from the same farm, which was subjected to the same feeding and care conditions. While the agent could not be detected on the seventh day in the swab samples taken at regular intervals starting from the first week of May, a significant increase was found in colonization in parallel with the increasing age ($P < 0.05$) (Fig. 1).

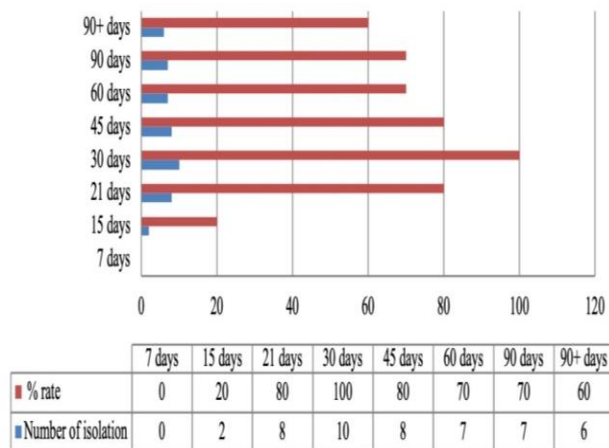


Fig. 1: Daily age-related cloacal colonization of the thermophilic *Campylobacter*

Molecular identification

As a result of mPCR, 151 (96.2%) thermophilic *Campylobacter* isolates were identified as *C. jejuni* and 6 (3.8%) as *C. coli*, which was 100% consistent with the identification findings by the phenotypical methods (Fig. 2).

Mass spectrometry

MALDI-TOF MS analysis was performed on only 125 isolates that could be sub-cultured. As a result of the analysis, 119 (95.2%) of the isolates were identified as *C. jejuni* and 6 (4.8%) as *C. coli* with a confidence interval of 99.9% (Appendix 1). MALDI-TOF MS identification findings were 100% compatible with the results of cultural analysis and mPCR (Table 3).

Antibiotic susceptibility test

Antibiotic susceptibility test of 157 isolates, including 151 *C. jejuni* and 6 *C. coli*, was performed with ampicillin, tetracycline, ciprofloxacin, gentamicin, and azithromycin. In total, resistance to different antibiotics was determined in 144 isolates. The highest resistance was determined to ciprofloxacin with 118 isolates (116 *C. jejuni* and 2 *C. coli*). Tetracycline resistance was determined in 65 isolates (63 *C. jejuni* and

2 *C. coli*), ampicillin resistance in 53 (50 *C. jejuni* and 3 *C. coli*), gentamicin resistance in 19 (*C. jejuni*), and azithromycin resistance in 12 isolates (*C. jejuni*). *C. jejuni* isolates were found mostly resistant to ciprofloxacin (76.8%), while azithromycin was the more effective antibiotic. *C. coli* isolates were found mostly (50%) resistant to ampicillin, while 100% sensitivity was determined against gentamicin and azithromycin (Table 4).

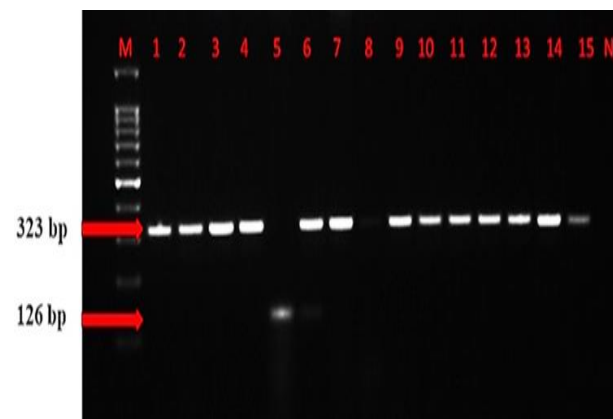


Fig. 2: Electrophoresis of the mPCR analyses of the thermophilic *Campylobacter* species. M: DNA ladder (Hyperladder 100 bp plus/Bioline), and N: Negative control. Lanes 1-15: Thermophilic *Campylobacter* field isolates. Lane 5 shows a *C. coli* isolate with 126 bp and the others show *C. jejuni* isolates with 323 bp

Resistance gene analysis

The resistance gene ratios of thermophilic *Campylobacter* isolates are shown in Table 5 using phenotypic and molecular (PCR) assays. Among the thermophilic *Campylobacter* species, the most common resistance gene was *tetO* with 90.8% frequency, followed by *aphA-3* with 52.7%, *gyrA* with 51.7%, and *blaOXA-61* with 35.2% (Fig. 3). The isolates were negative for the *ermB* gene.

Multiple drug resistance patterns

In the study, isolates with resistance to two or more antibiotics were evaluated as “multi-drug resistance (MDR)”. Among the thermophilic *Campylobacter* isolates total MDR rate was found as 52.87%. Double MDR was observed in 56 isolates (53 *C. jejuni* and 3 *C. coli*) such as TET-AMP in 5 (4 *C. jejuni* and 1 *C. coli*), CIP-AZM in 3 *C. jejuni*, GEN-AMP in 1 *C. jejuni*, CIP-AMP in 18 (16 *C. jejuni* and 2 *C. coli*), CIP-GEN in 2 *C. jejuni*, and TET-CIP in 27 *C. jejuni* isolates. Triple

Table 4: Antibiotic susceptibility profiles of the thermophilic *Campylobacter* isolates

| Antibiotic | <i>C. jejuni</i> | | Kruskal-Wallis P-value | <i>C. coli</i> | | Kruskal-Wallis P-value | Total | | Kruskal-Wallis P-value |
|---------------|------------------|------|------------------------|----------------|------|------------------------|-------|------|------------------------|
| | n:151 | % | | n:6 | % | | n:157 | % | |
| Ampicillin | 50 | 3.1 | P<0.05 | 3 | 50 | P<0.05 | 53 | 33.8 | P<0.05 |
| Tetracycline | 63 | 41.7 | | 2 | 33.3 | | 65 | 41.4 | |
| Ciprofloxacin | 116 | 76.8 | | 2 | 33.3 | | 118 | 75.2 | |
| Gentamicin | 19 | 12.6 | - | - | 19 | 12.1 | - | - | |
| Azithromycin | 12 | 7.9 | - | - | 12 | 7.6 | - | - | |

Table 5: Resistance gene profiles of the thermophilic *Campylobacter* isolates obtained by PCR

| Gene | Phenotypically resistant isolates | PCR positivity of resistance gene | | Phenotypically resistant <i>C. jejuni</i> | PCR positivity of resistance gene of <i>C. jejuni</i> isolates | | | Phenotypically resistant <i>C. coli</i> | PCR positive of resistance gene of <i>C. coli</i> isolates | | |
|-----------------------------|-----------------------------------|-----------------------------------|------------------------|---|--|------|------------------------|---|--|----|------------------------|
| | | n (%) | Kruskal-Wallis P-value | | n | % | Kruskal-Wallis P-value | | n | % | Kruskal-Wallis P-value |
| | | | | | | | | | | | |
| <i>bla_{OXA-61}</i> | 53 | 19 (35.2) | P<0.05 | 50 | 17 | 34 | P<0.05 | 3 | 2 | 66 | - |
| <i>tetO</i> | 65 | 59 (90.8) | P<0.05 | 63 | 58 | 98.3 | P<0.05 | 2 | 1 | 50 | - |
| <i>gyrA</i> | 118 | 60 (51.7) | P<0.05 | 116 | 59 | 50.8 | P<0.05 | 2 | 1 | 50 | - |
| <i>aphA-3</i> | 19 | 10 (52.7) | P<0.05 | 19 | 10 | 52.7 | P<0.05 | - | - | - | - |
| <i>ermB</i> | 12 | - | - | 12 | - | - | - | - | - | - | - |

**Fig. 3:** Gel electrophoresis image of PCR analysis of resistance genes. M: DNA ladder (HyperLadder 1 kb plus/Bioline), and N: Negative control. Lanes 1-3: *gyrA* gene with 620 bp, Lanes 4-6: *tetO* gene with 559 bp, Lanes 7-9: *bla_{OXA-61}* gene with 372 bp, and Lanes 10-13: *aphA-3* gene with 701 bp**Table 6:** Multi-drug resistance (MDR) profiles of the thermophilic *Campylobacter* isolates

| MDR profile | <i>C. jejuni</i> | <i>C. coli</i> | Total |
|---------------------|------------------|----------------|-------|
| TET& | 4 | 1 | 5 |
| CIP&AZM | 3 | - | 3 |
| GEN& | 1 | - | 1 |
| CIP& | 16 | 2 | 18 |
| CIP&GEN | 2 | - | 2 |
| TET&CIP | 27 | - | 27 |
| CIP&&AZM | 1 | - | 1 |
| CIP&GEN& | 2 | - | 2 |
| TET&CIP& | 10 | - | 10 |
| TET&GEN& | 1 | - | 1 |
| TET&CIP&GEN | 5 | - | 5 |
| TET&CIP&GEN&AZM | 1 | - | 1 |
| TET&CIP&GEN& | 4 | - | 4 |
| TET&CIP&&AZM | 2 | - | 2 |
| TET&CIP&AZM&&GEN | 1 | - | 1 |
| Total | 80 | 3 | 83 |

MDR was observed in 19 *C. jejuni* isolates such as CIP-AMP-AZM in 1, CIP-GEN-AMP in 2, TET-CIP-AMP in 10, TET-GEN-AMP in 1, and TET-CIP-GEN in 5 isolates. Quadruple MDR was observed in 7 *C. jejuni* isolates such as TET-CIP-GEN-AZM in 1, TET-CIP-GEN-AMP in 4, and TET-CIP-AMP-AZM in 2 isolates. Only one quintette MDR was observed in one *C. jejuni* and this isolate was almost assumed as “superbug” (Table 6).

Discussion

Cloacal carriage in poultry and subsequent scattering with faeces is an important epidemiological feature of

the thermophilic *Campylobacter* for both human and animal infections. In studies, the prevalence of the thermophilic *Campylobacter* in cloacal swab or fecal samples of geese varies between 35% and 89.6% (Aydin *et al.*, 2001; Elmalı *et al.*, 2004; Colles *et al.*, 2008; Moriarty *et al.*, 2011). The prevalence of the thermophilic *Campylobacter* of the current study was found to be 39.3% in goose and showed a big harmony with the others (Aydin *et al.*, 2001; Colles *et al.*, 2008; Moriarty *et al.*, 2011). However, the main reasons for the difference (Elmalı *et al.*, 2004) can be caused by factors such as geographical, climate, and seasonal differences, cultivation style, and age ranges of sampled animals.

Intestinal colonization of the thermophilic *Campylobacter* in poultry increases in parallel with the age and is lower in birds younger than 2-3 weeks due to the presence of maternal antibodies (Sahin *et al.*, 2003) and natural gut flora (van der Wielen *et al.*, 2000). This hypothesis is also confirmed by experimental studies (Newell, 2001; Boyd *et al.*, 2005). In this study, while the thermophilic *Campylobacter* were not found in 7-day-old goslings, the cloacal colonization started on day 15 and reached 100% at day 30. These results were compatible with the results of studies indicating persistent negativity of the cloacal colonization until at least 10 days of age that was the so-called lag phase (Newell and Fearnley, 2003; Sahin *et al.*, 2003). Similarly, a biased increase occurs in cloacal colonization towards the warmer months (May to October) in poultry, and this leads a similar seasonal course in human infections (Leflon-Guibout and Munier, 2016; Kalupahana *et al.*, 2018; Es-Soucratti *et al.*, 2020). The highest colonization rate was found in summer (58.3%) followed by spring (39.2%) months in the present study. This is in parallel with reports suggesting that increased cloacal colonization is likely due to several reasons, such as increased frequency of environmental exposure of birds and free access to water resources (Jorgensen *et al.*, 2011; Leflon-Guibout and Munier, 2016).

PCR in precise diagnosis and differentiation of the thermophilic *Campylobacter* species has been widely used. In this study, the compatibility of the PCR with the phenotypical methods in species differentiation was found to be similar to the reported studies. In addition, the dominance (96.2%) of *C. jejuni* is also evident (Szosland-Faltyn *et al.*, 2008; Wysok *et al.*, 2020). Compared to the other methods, MALDI-TOF mass spectrometry is more advantageous in terms of speed and cost and enables a novel diagnostic approach (Seng *et al.*, 2009). MALDI-TOF MS has also been used to

identify *Campylobacter* species and has demonstrated high compatibility with PCR (Scerbova and Laukova, 2016; Nowaczek *et al.*, 2019). In this study, MALDI-TOF MS analysis was performed for 125 isolates due to their high fragility in subcultures in which 119 (95.2%) were identified as *C. jejuni* and 6 (4.8%) as *C. coli* with 100% agreement with other tests. This high diagnostic agreement among identification methods is similar to that reported for the thermophilic *Campylobacter* species (Scerbova and Laukova, 2016; Nowaczek *et al.*, 2019).

The benign course of *Campylobacter* infections depends on applications both rapid diagnosis of the agent and accurate treatment of the infection. Birds do not require treatment because of their asymptomatic or mild clinical course. However, knowing the etiological and antibiotic susceptibility characteristics of the causative agent circulated in poultry can offer an insight into human beings. Macrolides, aminoglycosides, tetracyclines, fluoroquinolones, and beta-lactams are widely used in treatment of the thermophilic *Campylobacter* infections (Allos, 2001). Bacterial resistance to antibiotics is mostly caused by the genes encoded by plasmid or chromosomal DNA and their prevalence varies according to determinants such as host and environmental conditions. In this study, the rates of resistance and the prevalence of the common resistance gene for these antibiotics were either within the previously reported ranges (Aydin *et al.*, 2001; Griggs *et al.*, 2009; Bardoň *et al.*, 2017; Szczepanska *et al.*, 2017; Marotta *et al.*, 2020; Proietta *et al.*, 2020; Wysok *et al.*, 2020) or very close (Aydin *et al.*, 2001; Bakeli *et al.*, 2008; Çokal *et al.*, 2009; Jamali *et al.*, 2013; Abdi-Hachesoo *et al.*, 2014; Bardoň *et al.*, 2017; Bailey *et al.*, 2019; Melo *et al.*, 2019; Silva *et al.*, 2019). Indiscriminate use of antibiotics in the treatment of clinically important bacterial infections causes multidrug resistance. Multiple resistance profiles have been reported against many antimicrobial agents from different classes, such as fluoroquinolones, beta-lactams, tetracyclines, aminoglycosides, and chloramphenicol among the thermophilic *Campylobacter* species (Hasdemir, 2007; Bardoň *et al.*, 2017; Wysok *et al.*, 2020). This resistance, which can occur in dual or more combinations and in different antibiotic groups, varies between 28% and 90.7% (Bardoň *et al.*, 2017; Wysok *et al.*, 2020). In this study, multiple antimicrobial resistance was found to be 57.6% for *C. jejuni* and 50% for *C. coli*, within the same rates and profiles reported in other studies. It is thought that the proportional differences may be caused by the type of sample taken, the sample area and the diversity of the antimicrobials used.

In conclusion, phenotypic methods, PCR and MALDI-TOF MS were used together to identify thermophilic *Campylobacter* species and diagnostic values were found to be quite compatible with each other. It is thought that the MALDI-TOF MS, which offers a high (99.9%) confidence interval and high sensitivity in a few hours, may be a technique that can be applied in the diagnosis of pathogens in the field of veterinary science. Azithromycin and gentamicin

resistance was found to be low among the thermophilic *Campylobacter* isolates, while resistance to others was quite high. Identification of genes responsible for the resistance to related antibiotics is important in terms of revealing the epidemiology of resistance, developing appropriate treatment strategies, and planning preventive measures correctly.

Acknowledgements

This research was supported by the Research Fund of Kafkas University with a Project No: 2019-TS-50. The authors would like to thank Prof. B. Otlu from the İnönü University Faculty of Medicine (Turkey) for his help on MALDI-TOF MS analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Abdi-Hachesoo, B; Khoshbakht, R; Sharifiyazdi, H; Tabatabaei, M; Hosseinzadeh, S and Asasi, K (2014). Tetracycline resistance genes in *Campylobacter jejuni* and *C. coli* isolated from poultry carcasses. *Jundishapur J. Microbiol.*, 7: e12129.
- Allos, BM (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin. Infect. Dis.*, 32: 1201-1206.
- Anonymous 1 (2020). www.mikrobiology.org/TR. (available at 12.08.2020).
- Aydin, F; Atabay, HI and Akan, M (2001). The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). *J. Appl. Microbiol.*, 90: 637-642.
- Bailey, MA; Taylor, RM; Brar, JS; Corkran, SC; Velásquez, C; Rama, EN; Oliver, HF and Singh, M (2019). Prevalence and antimicrobial resistance of *Campylobacter* from antibiotic-free broilers during organic and conventional processing. *Poult. Sci.*, 98: 1447-1454.
- Bakeli, G; Sato, K; Kumita, W; Saito, R; Ono, E; Chida, T and Okamura, N (2008). Antimicrobial susceptibility and mechanism of quinolone resistance in *Campylobacter jejuni* strains isolated from diarrheal patients in a hospital in Tokyo. *J. Infect. Chemother.*, 14: 342-348.
- Bardoň, J; Pudová, V; Koláčková, I; Karpíšková, R; Röderová, M and Kolář, M (2017). Virulence and antibiotic resistance genes in *Campylobacter* spp. in the Czech Republic. *Epidemiol. Mikrobiol. Imunol.*, 66: 59-66.
- Boyd, Y; Herbert, EG; Marston, KL; Jones, MA and Barrow, PA (2005). Host genes affect intestinal colonisation of newly hatched chickens by *Campylobacter jejuni*. *Immunogenetics*. 57: 248-253.
- Chopra, IT and Roberts, M (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.*, 65: 232-260.
- Çokal, Y; Caner, V; Sen, A; Çetin, C and Karagenc, N (2009). *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: An epidemiological survey study in Turkey. *Zoonoses Public Health*. 56: 105-110.

- Coker, AO; Isokpehi, RD; Thomas, BN; Amisu, KO and Obi, CL** (2002). Human *Campylobacteriosis* in developing countries. *Emerg. Infect. Dis.*, 8: 237-244.
- Colles, FM; Jones, TA; McCarthy, ND; Sheppard, SK; Cody, AJ; Dingle, KE; Dawkins, MS and Maiden, MCJ** (2008). *Campylobacter* infection of broiler chickens in a free-range environment. *Environ. Microbiol.*, 10: 2042-2050.
- Elmah, M; Yaman, H; Ulukanlı, Z and Genctav, K** (2004). Isolation and identification of *Campylobacter jejuni* in goose carcass, goose cloaca, goose bedding and goose, chicken and quail eggs. *Vet. Bil. Derg.*, 20: 47-52 (in Turkish with an abstr. in English).
- Es-Soucratti, K; Hammoumi, A; Bouchrif, B; Asmai, R; En-Nassiri, H and Karraouan, B** (2020). Occurrence and antimicrobial resistance of *Campylobacter jejuni* isolates from poultry in Casablanca-Settat, Morocco. *Ital. J. Food Saf.*, 9: 54-59.
- Evans, SJ** (1997). Epidemiological studies of *Salmonella* and *Campylobacter* in poultry. Ph.D. Thesis, University of London, London, United Kingdom. PP: 1-331.
- Evans, S and Sayers, AR** (2000). A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. *Prev. Vet. Med.*, 46: 209-223.
- Griggs, DJ; Peake, L; Johnson, MM; Ghorri, S; Mott, A and Piddock, LJ** (2009). Beta-lactamase-mediated beta-lactam resistance in *Campylobacter* species: prevalence of *Cj0299 (blaOXA-61)* and evidence for a novel beta-Lactamase in *C. jejuni*. *Antimicrob. Agents Chemother.*, 53: 3357-3364.
- Hasdemir, U** (2007). The role of cell wall organization and active efflux pump systems in multidrug resistance of bacteria. *Mikrobiyol. Bul.*, 41: 309-327 (in Turkish with an abstr. in English).
- Jamali, H; Ghaderpour, A; Radmehr, B; Wei, KSC; Chai, LC and Ismail, S** (2013). Prevalence and antimicrobial resistance of *Campylobacter* species isolates in ducks and geese. *Food Control*. 50: 328-330.
- Jones, K** (2011). *Campylobacters* in water, sewage and the environment. *J. Appl. Microbiol.*, 90: 68-79.
- Jorgensen, F; Ellis-Iversen, J; Rushton, S; Bull, SA; Harris, SA; Bryan, SJ; Gonzalez, A and Humphrey, TJ** (2011). Influence of season and geography on *Campylobacter jejuni* and *C. coli* subtypes in housed broiler flocks reared in Great Britain. *Appl. Environ. Microbiol.*, 77: 3741-3748.
- Kalupahana, RS; Mughini-Gras, L; Kottawatta, SA; Somarathne, S; Gamage, C and Wagenaar, JA** (2018). Weather correlates of *Campylobacter* prevalence in broilers at slaughter under tropical conditions in Sri Lanka. *Epidemiol. Infect.*, 146: 972-979.
- Komba, EV; Mdegela, RH; Msoffe, PL; Nielsen, LN and Ingmer, H** (2015). Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses Public Health*. 62: 557-568.
- Lambert, T; Gerbaud, G; Trieu-Cuot, P and Courvalin, P** (1985). Structural relationship between the genes encoding 3'-aminoglycoside phosphotransferases in *Campylobacter* and in Gram-positive cocci. *Ann. Inst. Pasteur Microbiol.*, 136B: 135-150.
- Leflon-Guibout, V and Munier, AL** (2016). *Campylobacter* infections: Epidemiology, virulence factors, antibiotic resistance. *J. Anti-Infect.*, 18: 160-168.
- Luangtongkum, T; Jeon, B; Han, J; Plummer, P; Logue, CM and Zhang, Q** (2009). Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. *Future Microbiol.*, 4: 189-200.
- Mamur, J** (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.*, 3: 208-218.
- Marotta, F; Janowicz, A; Marcantonio, LD; Ercole, C; Donato, GD; Garofolo, G and Giannatale, ED** (2020). Molecular characterization and antimicrobial susceptibility of *C. jejuni* isolates from Italian wild bird populations. *Pathogens*. 9: 1-16.
- Melo, RT; Graziotin, AL; Valadares-Júnior, EC; Prado, RR; Mendonça, EP; Monteiro, GP; Peres, PABM and Rossi, DA** (2019). Evolution of *Campylobacter jejuni* of poultry origin in Brazil. *Food Microbiol.*, 82: 489-496.
- Moriarty, EM; Karki, N; Mackenzie, M; Sinton, LW; Wood, DR and Gilpin, BJ** (2011). Faecal indicators and pathogens in selected New Zealand waterfowl. *N. Z. J. Mar. Fresh. Res.*, 45: 679-688.
- Newell, DG** (2001). Animal model of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar *Helicobacter pylori* models. *J. Appl. Microbiol.*, 90: 57S-67S.
- Newell, DG and Fearnley, C** (2003). Sources of *Campylobacter* colonization in broiler chickens. *Appl. Environ. Microbiol.*, 69: 4343-4351.
- Nowaczek, A; Urban-Chmiel, R; Dec, M; Puchalski, A; Stępień-Pyśniak, D; Marek, A and Pyzik, E** (2019). *Campylobacter* spp. and bacteriophages from broiler chickens: Characterization of antibiotic susceptibility profiles and lytic bacteriophages. *MicrobiologyOpen*. 8: e784.
- Proietti, PC; Guelfia, G; Belluccia, S; De Lucab, S; Di Gregorio, S; Pieramatia, C and Franciosi, MP** (2020). Beta-lactam resistance in *Campylobacter coli* and *Campylobacter jejuni* chicken isolates and the association between *blaOXA-61* gene expression and the action of β -lactamase inhibitors. *Vet. Microbiol.*, 241: 108553.
- Qin, S; Wang, Y; Zhang, Q; Zhang, M; Deng, F; Shen, Z; Wu, C; Wang, S; Zhang, J and Shen, J** (2014). Report of ribosomal RNA methylase gene erm(B) in multidrug-resistant *Campylobacter coli*. *J. Antimicrob. Chemother.*, 69: 964-968.
- Refregier-Petton, J; Rose, N; Denis, M and Salvat, G** (2001). Risk factors for *Campylobacter* spp. contamination in French broiler chicken flocks at the end of the rearing period. *Prev. Vet. Med.*, 50: 89-100.
- Ringo, DD and Korolik, V** (2002). Colonisation phenotype and colonization differences in *Campylobacter jejuni* strains in chickens before and after passage *in vivo*. *Vet. Microbiol.*, 92: 225-235.
- Sahin, O; Luo, N; Huang, S and Zhang, Q** (2003). Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. *Appl. Environ. Microbiol.*, 69: 5372-5379.
- Scerbova, J and Laukova, A** (2016). Sensitivity to enterocins of thermophilic *Campylobacter* spp. from different poultry species. *Foodborne Pathog. Dis.*, 13: 668-673.
- Seng, P; Drancourt, M; Gouriet, F; La Scola, B; Fournier, PE; Rolain, JM and Raoult, D** (2009). Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laserdesorption ionization time-of-flight mass spectrometry. *Clin. Infect. Dis.*, 49: 543-551.
- Shreeve, JE; Toszeghy, M; Pattison, M and Newell, DG** (2000). Sequential spread of *Campylobacter* infection in a multi-pen broiler house. *Avian Dis.*, 44: 983-988.
- Silva, PR; Palma, JM; Souza, NR; de Moura, HM; Perecmanis, S and Patricia-Santana, A** (2019). Isolation and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* found in chilled chicken carcasses in the Federal District Region and surrounding areas. *Semin.*

- Cienc. Agrár., 40: 2247-2260.
- Szczepanska, B; Andrzejewska, M; Spica, D and Klawe, J** (2017). Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiol.*, 17: 1-9.
- Szosland-Faltyn, A; Bartodziejska, B; Krolasik, J; Domanska, BP; Korsak, D and Chmiel, M** (2008). The prevalence of *Campylobacter* spp. in Polish poultry meat. *Pol. J. Microbiol.*, 67: 117-120.
- Taremi, M; Soltan-Dalla, MM; Gachkar, L; Moez-Ardalan, S; Zolfagharian, K and Zali, MR** (2006). Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. *Int. J. Food Microbiol.*, 108: 401-403.
- The Clinical & Laboratory Standards Institute (CLSI)** (2017). M 100: Performance Standarts for Antimicrobial Susceptibility Testing, 27th Edition, January 2017.
- van der Wielen, PWJJ; Biesterveld, S; Notermans, S; Hofstra, H; Urlings, BAP and van Knapen, F** (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Appl. Environ. Microbiol.*, 66: 2536-2540.
- Wang, G; Clark, CG; Taylor, TM; Pucknell, C; Barton, C; Price, L; Woodward, DL and Rodgers, FG** (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* and *C. fetus* subsp. *fetus*. *J. Clin. Microbiol.*, 40: 4744-4747.
- Wang, Y; Huang, WM and Taylor, DE** (1993). Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. *Antimicrob. Agents Chemother.*, 37: 457-463.
- Wieczorek, K and Osek, J** (2013). Antimicrobial resistance mechanisms among *Campylobacter*. *Biomed. Res. Int.*, 2013: 340605.
- Wysok, B; Wojtacka, J; Wiszniewska-Laszczych, A and Szteyn, J** (2020). Antimicrobial resistance and virulence properties of *Campylobacter* spp. originating from domestic geese in Poland. *Animals*. 10: 1-15.
- Yakupoğulları, Y; Otlı, B; Çelik, B and Gözükarı Bağ, HG** (2019). Performance of MALDI-TOF MS for the identification of Gram-negative bacteria grown on Eosin Methylene Blue (EMB) agar: A simple method for improving the effectiveness of identification. *Mikrobiyol. Bul.*, 53: 1-11 (in Turkish with an abstract in English).