Annals of Agrarian Science

Journal homepage: http://journals.org.ge/index.php

Campylobacter coli and *Campylobacter jejuni* in Georgian Retail Chicken: Isolation, Identification and Antibiotic Susceptibility

L. Ushanov*, I. Pipia, I. Abuseridze, A. Bagishvili, A. Meshvildishvili, N. Gagelidze

Agricultural University of Georgia

Received: 10 October, 2022; Accepted: 15 November, 2022

A B S T R A C T

Campylobacter species have been presently recognized as the most frequent cause of enteric infections worldwide. To date, the prevalence and significance of *Campylobacter* infections in Georgia have not been assessed. This study aims to partially address this circumstance and provide some information on the prevalence of *Campylobacter* species in retail chicken meats sold in Tbilisi supermarkets. A total of 200 chicken meats were purchased during a two-year period. The purchased meat samples represented 6 different Georgian chicken meat producers. After isolation, 74 samples (37 %) were found to be harboring either *Campylobacter jejuni* or *Campylobacter coli,* as confirmed by MALDI TOF mass spectrometry. The isolated *C. jejuni* and *C. coli* strains were tested for antibiotic susceptibility by the disc diffusion method. *C. jejuni* and *C. coli* demonstrated high resistance to several types of antibiotics, such as ampicillin (28 % and 51 %, respectively), ciprofloxacin (79 % and 97 %, respectively) and tetracycline (28 % and 51 %, respectively).

This study concludes that 37 % of Georgian chicken meat harbors *Campylobacter* species. Most certainly, the real rate of contamination of chicken meat with these microorganisms is much higher, due to the difficulty of their isolation. Phenotypically, the local *C. jejuni* isolates differ from those of *C. jejuni* ATCC 33560.

Further studies are needed to show the clonality of the *Campylobacter* isolates, as well as their association with the diarrheal disease among patients diagnosed with enteric infections in Georgia.

Key words: *Campylobacter jejuni*, *Campylobacter coli*, Georgian Retail Chicken.

*Corresponding author: Leonid Ushanov, Email: l.ushanov@agruni.edu.ge

Introduction

Campylobacter spp. are short and motile, curved microaerobic Gram-negative rods common to many different animal hosts including livestock, wild animals and pets (Battersby et al., 2016). In humans, *Campylobacter* infections are strongly associated with the consumption of contaminated chicken meat, although there are other ways of acquiring *Campylobacter* infections (Kinana et al., 2007). Out of 39 *Campylobacter* species identified to date, the most frequently isolated and clinically important species are *C. jejuni* and *C. coli,* which share 86.6 % of the genome (Kinana et al. 2007).

Infections due to *Campylobacter* spp. have been on the rise worldwide during the past two decades, along with the resistance of *Campylobacter* spp. to various antibiotics (Agunos et al., 2014). Occurrence of campylobacter-related enterocolitis in the world amounts to 400-500 million cases yearly (Vlieghe et al., 2008). It has also been estimated that worldwide 50 % of chicken meat is contaminated with *Campylobacter* spp. (Vandeputte et al., 2019).

Poultry has been recognized as the major reservoir of *Campylobacter* spp. (Connerton et al., 2018), (Hakeem & Lu, 2021). Prevalence of *Campylobacter spp.* in raw farm-raised chicken meat varies from country to country, however up to 100 % of birds may harbor this pathogen at slaughter

age. In poultry, colonization with *Campylobacter* spp. is not associated with any clinical signs. These microorganisms are localized mostly in the cecum and the cloacal crypt of birds (Le et al., 2012). Various organs, such as the liver, the small intestine, and gizzard usually also contain this pathogen (Epps et al., 2013). Contamination events with *Campylobacter* spp. occur during slaughter, leading to cross-contamination of the carcasses with rates as high as 100% (Hakeem & Lu, 2021).

In the past scientists ascribed the diversity of *C. coli* and *C. jejuni* strains to their abundance in wild birds and animals. Increasing evidence, however, suggests that anthropogenic factors are the driving force in the evolution of these microorganisms (Sheppard & Maiden, 2015). Industrial farms, with extremely large numbers of broilers, opened a novel niche for *Campylobacter* spp. resulting in the emergence of *C. jejuni* lineages able to colonize multiple hosts. In parallel, expansion of particular *C. coli* lineages common to both agricultural animals and sick humans have been taking place along with the emergence and proliferation of resistant lineages of *C. jejuni* and *C. coli* with genetic exchange between these lineages (Sheppard & Maiden, 2015).

Although most of human campylobacteriosis cases are self-limiting, complications may occur as post-infection sequelae (Yang et al., 2019). Seriously debilitating post-infection complications include Guillain Barre syndrome, Reiter's syndrome and Irritable Bowel Syndrome (IBS). Additionally, children, the elderly and immunocompromised patients may suffer complications resulting from *Campylobacter* infections (Facciolà et al., 2017).

In most developing countries, costly reagents as well as difficulties of culturing *Campylobacter* spp. make the isolation and identification of these microorganisms challenging (Ghorbanalizadgan et al., 2019). Often identification of *Campylobacter* spp. is not a part of the routine laboratory workup. For these reasons, many cases of human campylobacteriosis in developing countries are frequently misdiagnosed.

An equally important issue is the uncontrolled use of antibiotics and antimicrobial substances in the meat and other agriculture industries (Serwecińska, 2020). Whether the particular poultry farms involved in this study engage in the use of antibiotics for sub-therapeutic purposes, could not be confirmed. However, judging from the high levels of resistance observed against penicillin, fluoroquinolones and tetracyclines among the isolated *Campylobacter* spp., it may be concluded that birds are frequently exposed to these substances in local poultry farms (Yang et al., 2019).

Materials and Methods

Sample Collection

Samples were collected over a two-year period, from fall 2018 to fall 2020. During this time chicken carcasses, chicken livers, chicken breasts and thighs from various meat producers were purchased in Tbilisi supermarkets. The samples, as packaged, were placed in clean plastic bags and kept in a cooler bag until needed. Usually, the purchased samples were processed for the isolation of *Campylobacter* spp. within 2-3 hours from purchase. One separate chicken carcass or a single package of chicken livers were treated as one isolation instance. Overall, about 200 samples of 6 different producers were purchased resulting in 74 isolates. Seasonality was not taken as a factor in this study.

Isolation and Culturing of *Campylobacter*

Sampling

Chicken breasts and thighs were sampled with sterile cotton swabs. Chicken carcasses were sampled in multiple areas. The swabs were then placed for enrichment into sterile 15 mL conical tubes containing 5 mL of buffered peptone water. At the same time, parts of the skin (about 15 g of the neck, wing, and thigh or rump area) were removed and placed for enrichment into a sterile 50 mL conical tube containing 25 mL of buffered peptone water. Chicken livers were placed in a sterile petri dish and minced with a sterile lancet, after which the minced portion of the liver was transferred into a 15 mL tube with peptone water for the enrichment step.

Enrichment Media and Conditions

Samples were enriched using buffered peptone water (pH 7.0), (Biolife, Italy). The enrichment time did not exceed 2 hours. The incubation temperatures of 37°C and 42°C were tested prior to conducting isolations of *Campylobacter* spp. (data not shown). The optimal growth temperature for isolation of *Campylobacter* spp. was determined to be 37°C (data not shown).

Culture Media

Dehydrated Cefoperazone Charcoal Deoxicholate Agar/mCCDA/Campylobacter Blood Free Medium Base, Bolton (Biolife, Italy) was used for inoculating the enriched samples. Campylobacter Selective Supplement (Liophilchem, Spain) containing cefoperazone (16 mg/L) and amphotericin B (5 mg/L) was added into the medium after cooling off to 50°C, according to the manufacturer's instructions (Liofilchem, Italy). Samples were inoculated at 50 uL per plate using the four-quadrant isolation method. Sample inoculation on mCCDA agar plates was performed in quadruplicates, to increase the chance of isolation.

Culturing Conditions

37°C and 42°C temperatures were tested for culturing *Campylobacters*. Candles and anaerobic jars were used to create microaerobic atmosphere. It was determined that 37°C worked better for isolation of *Campylobacter* spp. 42°C temperature was used for subculturing isolated strains.

Identification of *Campylobacter* spp.

Staining:

Initially, Gram's staining kit (Deltalab, Italy) was used and then a single 1 % carbol fuchsin (Deltalab, Italy) stain to rapidly identify presumptive colonies of *Campylobacter* spp.

Confirmation by Campylobacter Latex Agglutination Test

Catalase activity and Campylobacter Latex Agglutination Test (Liofilchem, Italy) were used to confirm suspect colonies as *Campylobacter* spp. *C. jejuni* ATCC 33560 strain served as positive control.

Subculturing

Colonies that morphologically and microscopically resembled those of *Campylobacter* spp. were carefully picked with the blunt end of a sterile 10 µL inoculation loop, transferred onto the first quadrant of the CCDA agar plate and then inoculated on four quadrants. The inoculation loop was sterilized in between each quadrant. Each strain was subcultured from a discreet colony until pure culture was obtained (usually 2-3 times).

MALDI-TOF Mass Spectrometry

Campylobacter species were identified by Mass Spectrometry performed on Matrix Assisted Laser Desorption Ionization Time of Flight mass spectrometer (MALDI-TOF MS; Vitek-MS, Biomérieux, Nürtingen, Germany) at the Institute for Medical Microbiology and Hospital Hygiene of the University Hospital, Magdeburg.

Briefly, bacterial cultures were plated on Columbia blood agar (Oxoid) and incubated under microaerophilic conditions. After 48 hours, a single bacterial colony from a monoculture was used for identification. For this purpose, a colony was touched very lightly with the tip of a sterile toothpick and spread upon predefined spot on a barcoded slide with 48 spots, which were first pre-treated with 5 µL of the matrix solution. After all samples were transferred on the slide, it was run on the Mass-Spectrometer.

Antibiotic Susceptibility Testing

Kirby Bauer disk diffusion method was used to determine antibiotic susceptibility of the *Campylobacter* spp. Testing was performed on all confirmed *Campylobacter* isolates and interpreted according to the guidelines provided by 2022 European Committee on Antimicrobial Susceptibility (EUCAST).

Antibiotic disks (Oxoid) were placed on Mueller Hinton Agar plates supplemented with 5 % sheep's blood and inoculated with 0.5 McFarland standard of each respective bacterial strain monoculture. The susceptibility plates were incubated for 48 h at 37°C under microaerophilic conditions, after which the inhibition zones were measured. The zone diameters were interpreted as susceptible (S), or resistant (R) after the EUCAST guidelines (Paintsil et al., 2021).

Isolates resistant to at least one antibiotic from each of the following antimicrobial groups - tetracyclines, macrolides, and quinolones - were considered multi-drug resistant (MDR), which is defined as resistance to three or more antimicrobials of any substance group.

Table 1. *Breakpoints for Determination of Antibiotic Resistance of Campylobacter Isolates - C. coli and C. jejuni**

Antibiotic (disk concentration)	Zone Diameter (mm)	
	S≥	R<
Tetracycline (30 µg)	30	30
Ciprofloxacin (5 µg)	50	26
Erythromycin (15 µg) *C. coli*	20	20
Erythromycin (15 µg) *C. jejuni*	24	24
Ampicillin (10 µg)	13*	7*
Chloramphenicol (30 µg)	18*	18*
Kanamycin (30 µg)	15*	7*
Streptomycin (25 µg)	22*	13*

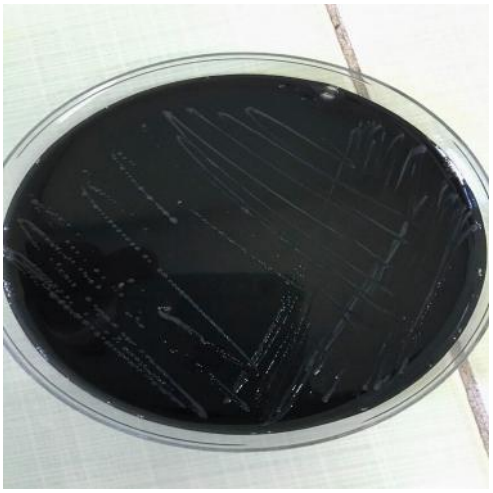

Figure 1. *Campylobacter spp. Isolate on a CCDA Plate*

Catalase Test

All suspect colonies displayed somewhat delayed catalase activity. Data not shown.

Latex Agglutination Test

The latex agglutination test kit was intended to identify *C. jejuni* isolates. This test was used at the initial stage to confirm the suspect colonies and it was observed that all suspect colonies resulted in agglutination, as compared to the positive control. Data not shown.

*Speciation of the **Campylobacter** Isolates using MALDI TOF Mass Spectrometry*

By using Mass Spectrometry analysis, 74 isolates that were presumed to belong to *Campylobacter* spp. were identified as *C. jejuni (*n=39) and *C. coli* (n=35).

Results

Colony Appearance

On CCDA agar *Campylobacter jejuni* colonies were observed as large, off-white, droplet-like or irregular-shaped and elongated, mucoid colonies. *Campylobacter coli* colonies were often observed as grayish-brown, oval-shaped or rounded, discrete, medium- to- smaller sized colonies. However, intermediate looking colonies were often observed as well.

Microscopy

Microscopy of the isolated strains revealed fine, pleomorphic, gram-negative rods. Staining of the local *Campylobacter* isolates did not reveal the classic "seagull wing" shape. Rather, the stained bacteria appeared either having a somewhat elongated and serpentine shape, or having a shorter, comma-like or "S"-shape appearance.

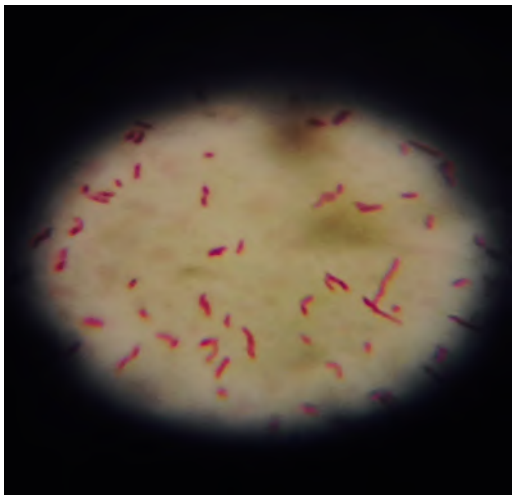

Figure 2. *Campylobacter spp. Isolate Stained with Carbol Fuchsin*

Table 2. *Speciation of Campylobacter Isolates using MALDI TOF Mass Spectrometry*

ID-ed Species	N of Isolates	Prevalence
C. jejuni	39 isolates	19.5 %
C. coli	35 isolates	17.5 %

Antibiotic Susceptibility Testing

Among the 35 isolates of *C. coli*, the highest resistance was observed to ciprofloxacin (97. 14 %), ampicillin (51.43 %), and tetracycline (51.43 %). One strain (CC- 20) was also resistant to both streptomycin and chloramphenicol (2.86 %).

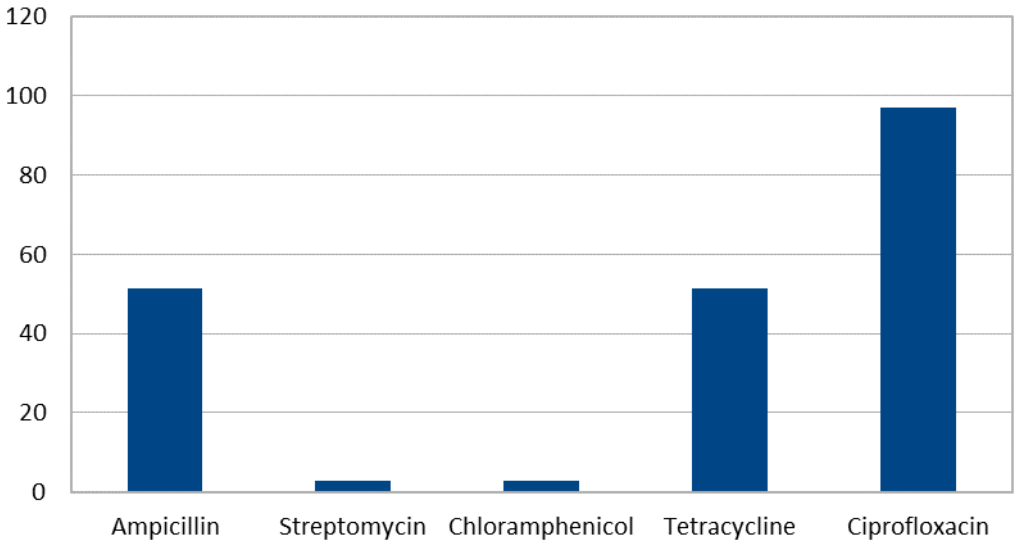

Figure 4. % Resistance of *C. jejuni* Isolates to Various Antibiotics

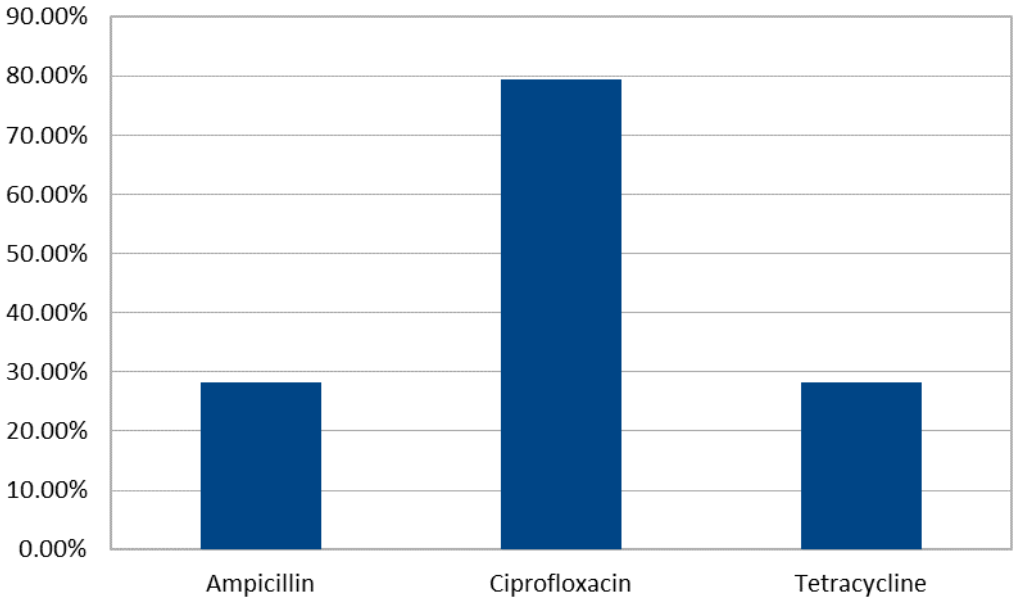

Figure 3. % Resistance of *C. coli* Isolates to Various Antibiotics

Resistance of the *C. jejuni* isolates to the same antibiotics appeared somewhat lower, however still at alarmingly high percentages: 28 % of the isolates were resistant to ampicillin and tetracycline, while 79 % showed resistance to ciprofloxacin.

As a result, 60 % of *C. coli* isolates and 28 % of *C. jejuni* isolates demonstrated resistance to at least three different classes of antibiotics, and thus qualifying as MDR (multi drug resistant) strains.

Discussion

This study is the first example of isolation of *Campylobacter* spp. from Georgian retail chicken meats. Various published methods, notably that of Oyarzabal et al., recommend mCCDA agar and CCDA antibiotic supplement containing cefoperazone and amphotericin B, for isolation of *Campylobacter* spp. (Oyarzabal et al., 2013).

The abundance of co-contaminant bacterial species made isolation of *Campylobacter* challenging in this study. Firstly, the antibiotic supplement did not inhibit many Gram-positive and some Gram-negative bacterial species. Secondly, allowing 48 h enrichment time resulted in the inhibition of slower-growing *Campylobacter* spp. by the faster-growing co-contaminant bacteria that also thrived in microaerobic conditions and 42°C. Thus, to limit the unwanted growth of irrelevant species, 37°C was used as the enrichment temperature, while the enrichment time was cut down to 2 hours.

Since Bolton broth also caused exuberant growth of irrelevant bacteria, sample enrichment was performed using buffered peptone water. It was essential that meat samples were completely submerged in the liquid. Vortexing samples several times during the enrichment step and prior to inoculation was also important to dislodge the bacteria from meat. 50 μL of sample was inoculated onto four different mCCDA agar plates after incubation.

Microscopy required some optimization as well. Initially, every suspect colony was stained using the Gram's method. Due to the fact that safranin stains *Campylobacter* poorly, resulting in bad microscopic visualization of the sample, we used another, fast direct staining method described by Mushi et al. for quickly identifying *Campylobacter* colonies. As a result, smears of suspect colonies were stained for 30 sec with 1 % carbol fuchsin and immediately examined for the presence of curved or pleomorphic rods (Mushi et al. 2014). The positive colonies were subcultured until pure culture was obtained. This technique saved a lot of time during the process of screening bacterial colonies.

Biochemically, *Campylobacter* spp. are relatively inert and they poorly hydrolyze sugars. To distinguish between species, scientists rely on a few biochemical characteristics (Burnett et al., 2002). One such biochemical test is identification of *C. jejuni* by its ability to hydrolyze hippurate. However, about 10% of *C. jejuni* isolates are incapable of hydrolyzing hippurate. Moreover, there is another, hippuricase-positive species-*C. avium,* first isolated in Italy in 2006 from chicken (Miller et al., 2017). To avoid confusing results, for species identification purposes we used MALDI TOF mass spectrometry. This method relies on databases of previously run and validated profiles of microorganisms and delivers results that are almost 100 % accurate. University of Magdeburg's Laboratory of medical Microbiology and Hygiene kindly provided us with this service.

Proportions of the poultry isolates of *C. jejuni* and *C. coli* among *Campylobacter* spp. isolated in a specific country vary geographically. In Canada, for example, *C. jejuni* dramatically outnumbered *C. coli* among *Campylobacter* spp. isolated from chicken: 87 % vs. 12 % (Dramé et al., 2020). In a Brazilian study, all 87 % of the total *Campylobacter* isolates turned out to be *C. jejuni* exclusively (Rodrigues et al., 2021). In other countries *C. coli* was isolated at the rates equal to or exceeding those of *C. jejuni*. For example, a recent Australian study found that the majority of *Campylobacter* isolates from fresh and frozen chicken carcasses and meats were those of *C. coli* (50-77%), whereas *C. jejuni* isolates were more common (50-88%) in beef, pork and lamb (Walker et al., 2019). A 2020 Chinese study identified *C. jejuni* and *C. coli* at almost equal numbers (233 and 231, respectively) among the 464 isolates of *Campylobacter* spp (Tang et al., 2020). A 2016 Italian study identified more *C. coli* than *C. jejuni* among their campylobacter isolates (Pergola et al., 2017). A higher percentage (75.5 %) of *C. coli* was also identified among the *Campylobacter* isolates from chicken meat compared to *C. jejuni* (24.5 %) in a 2011-2013 Polish study (Szczepanska et al., 2017). However, another Polish study concluded that *C. jejuni*, not *C. coli,* was the predominant *Campylobacter* species in Polish poultry meats (Szosland-Fałtyn et al. 2018). A similar 2015 study from Thailand also identified more *C. coli* (n=94) than *C. jejuni* (n=36) among the isolates from samples taken in and around chicken farms and hatcheries (Thomrongsuwannakij et al., 2017) and an Argentinian study of 2011-2013 found that *C. jejuni* outnumbered *C. coli* both in kosher (36% to 2 %) and conventional (26 % to 4 %) meats. This finding agreed with previous studies conducted in Argentina where *C. jejuni* isolates significantly outnumbered those of *C. coli* (Guirin et al., 2020).

Thus, the fact that the prevalence of *C. coli* and *C. jejuni* in Georgian chicken is somewhat equal is not unusual and agrees with reports from other research groups.

Due to the widespread practice of antibiotic use in food animals in the past decades in order to prevent and control infections, or even to enhance the growth of food animals, *C. jejuni* and *C. coli* have often been reported as resistant to penicillins (Wieczorek & Osek, 2013). In this study too, all *Campylobacter* isolates were resistant to penicillin G. However, resistance to ampicillin was somewhat lower among *C. jejuni* isolates compared to C. *coli* (28 % vs. 53 %). These findings are similar to a 2011 Irish study, which detected resistance to ceftifur (58 %), ampicillin (25 %) and nalidixic acid (17 %) among *C. jejuni* strains (Madden et al., 2011).

Resistance of *Campylobacter* spp. to tetracycline is also frequently reported worldwide, since tetracycline is the most widely used antibiotic in avian production due to its low cost (Wieczorek and Osek, 2013). In this study, 51 % of *C. coli* strains and 28 % of *C. jejuni* strains were found to be resistant to tetracycline. The tet(O) gene, which causes this resistance, seems to have global presence and

has been detected in many parts of the world. For example, an Irish research group reported that 100 % of the chicken isolates of thermophilic *Campylobacters* were harboring the tet(O) gene (Lynch et al., 2020). Resistance to tetracycline was also high-98 % and 56 %-among all *Campylobacter* isolates in a 2017 study conducted in Thailand, while most isolates were also MDR strains (Thomrongsuwannakij, Blackall, and Chansiripornchai, 2017).

Resistance to ciprofloxacin was the highest among the *Campylobacter* spp. isolated in this study. Similarly, Polish scientists reported that 91 % of *C. jejuni* isolates were resistant to this antibiotic (Wieczorek and Osek, 2013). An Indian research group observed that the highest rate of resistance among the *C. jejuni* isolates from chicken meat was to nalidixic acid and ciprofloxacin - 81.25 % and 63.46 %, respectively (Sathiamoorthi, T., Joseph Sahayarayan, J. and Arivoli, A., 2016). High resistance levels to tetracycline, ciprofloxacin and nalidixic acid (79.1 %, 72.1 % and 65.1 %, respec - tively) were observed in a 2017 Italian study as well (Pedonese et al., 2017). High incidence of fluoroquinolone resistance was seen among the isolates of both species (100 % and 98.9 % in *C. jejuni* and *C. coli*, respectively) in a study conducted in Thailand.

Antibiotic resistance finds its way to wild bird populations as well. For example, tetracycline and fluoroquinolone resistant strains of *C. coli* and *C. jejuni* were identified in storks by a 2015 Polish study (Szczepańska et al., 2015).

Our findings are in agreement with reported by other scientists from around the world, indicating at the growing tendency of antibiotic resistance among *Campylobacter* spp. An extremely worrisome trend is the emergence of MDR *Campylobacter* strains. As mentioned previously, many isolates of *Campylobacter* spp. from Georgian retail chicken have shown resistance to 3 antibiotics of different classes: penicillins, tetracyclines and fluoroquinolones. One *C. coli* isolate (CC-20) showed additional resistance to chloramphenicol (a macrolide drug) and streptomycin (an aminoglycoside drug).

Conclusion

Georgian retail chicken harbors at least two *Campylobacter* species, *C. jejuni* and *C.* coli, with high percentage of MDR strains among them. High resistance to Ciprofloxacin and Tetracycline point to circulation of these antibiotics in local farms. Previous scientific opinion that the majority (90 %) of the food-borne illnesses are associated with *C. jejuni,* may no longer be valid. Prevalence of certain *Campylobacter* spp. in types of meats and in human and animal hosts clearly varies geographically (Igwaran & Okoh, 2019). Therefore, it remains to be ascertained which *Campylobacter* species are associated more frequently with human campylobacter infections in Georgia.

Acknowledgments

This article was written as a result of the research conducted within the infrastructure of the Sustainable Agricultural and Food Systems (SAFS) Structured Doctoral Project, jointly offered by the University of Kassel (Germany) and the Agricultural University of Georgia. The research was funded by the Volkswagen Foundation and Shota Rustaveli National Science Foundation of Georgia (SRNSFG)–Contract no. 04/47.

References

[1] Agunos, A., Waddell, L., Léger, D., & Taboada, E. (2014). A Systematic Review Characterizing On-Farm Sources of Campylobacter spp. For Broiler Chickens. *PLOS ONE*, *9*(8), e104905. https://doi.org/10.1371/journal.pone.0104905

[2] Battersby, T., Walsh, D., Whyte, P., & Bolton, D. J. (2016). *Campylobacter* growth rates in four different matrices: Broiler caecal material, live birds, Bolton broth, and brain heart infusion broth. *Infection Ecology & Epidemiology*, *6*(1), 31217. https://doi.org/10.3402/iee.v6.31217

[3] Burnett, T. A., Hornitzky, M. A., Kuhnert, P., & Djordjevic, S. P. (2002). Speciating *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry and humans using six PCR-based assays. *FEMS Microbiology Letters*, *216*(2), 201–209. https://doi.org/10.1111/j.1574-6968.2002.tb11436.x

[4] Connerton, P. L., Richards, P. J., Lafontaine, G. M., O'Kane, P. M., Ghaffar, N., Cummings, N. J., Smith, D. L., Fish, N. M., & Connerton, I. F. (2018). The effect of the timing of exposure to Campylobacter jejuni on the gut microbiome and inflammatory responses of broiler chickens. *Microbiome*, *6*(1), 88. https://doi.org/10.1186/s40168-018-0477-5

[5] Dramé, O., Leclair, D., Parmley, E. J., Deckert, A., Ouattara, B., Daignault, D., & Ravel, A. (2020). Antimicrobial Resistance of *Campy-*

lobacter in Broiler Chicken Along the Food Chain in Canada. *Foodborne Pathogens and Disease*, *17*(8), 512–520. https://doi.org/10.1089/fpd.2019.2752

[6] Epps, S., Harvey, R., Hume, M., Phillips, T., Anderson, R., & Nisbet, D. (2013). Foodborne Campylobacter: Infections, Metabolism, Pathogenesis and Reservoirs. *International Journal of Environmental Research and Public Health*, *10*(12), 6292–6304. https://doi.org/10.3390/ijerph10126292

[7] Facciolà et al. (2017). Campylobacter: From microbiology to prevention. *J Prev Med Hyg.*, 58(2)(58(2)), 79–92.

[8] Francesca Pedonese, Roberta Nuvoloni, Barbara Turchi, Beatrice Torracca, Elisabetta Di Giannatale, Francesca Marotta and Domenico Cerri. (2017). *Prevalence, phenotypic and genetic diversity of Campylobacter in poultry fresh meat and poultry products on retail sale in Tuscany (Italy)*. *53*(1), 29–37.

[9] Ghorbanalizadgan, M., Bakhshi, B., Shams, S., & Najar-Peerayeh, S. (2019). Pulsed-field gel electrophoresis fingerprinting of Campylobacter jejuni and Campylobacter coli strains isolated from clinical specimens, Iran. *International Microbiology*, *22*(3), 391–398. https://doi.org/10.1007/s10123-019-00062-8

[10] Guirin, G. F., Brusa, V., Adriani, C. D., & Leotta, G. A. (2020). Prevalence of Campylobacter jejuni and Campylobacter coli from broilers at conventional and kosher abattoirs and retail stores. *Revista Argentina de Microbiología*, *52*(3), 217–220. https://doi.org/10.1016/j.ram.2019.07.002

[11] Hakeem, M. J., & Lu, X. (2021). Survival and Control of Campylobacter in Poultry Production Environment. *Frontiers in Cellular and Infection Microbiology*, *10*, 615049. https://doi.org/10.3389/fcimb.2020.615049

[12] Igwaran, A., & Okoh, A. I. (2019). Human campylobacteriosis: A public health concern of global importance. *Heliyon*, *5*(11), e02814. https://doi.org/10.1016/j.heliyon.2019.e02814

[13] Kinana, A. D., Cardinale, E., Bahsoun, I., Tall, F., Sire, J.-M., Breurec, S., Garin, B., Saad-Bouh Boye, C., & Perrier-Gros-Claude, J.-D. (2007). Campylobacter coli isolates derived from chickens in Senegal: Diversity, genetic exchange with Campylobacter jejuni and quinolone resistance. *Research in Microbiology*, *158*(2), 138–142. https://doi.org/10.1016/j.resmic.2006.11.009

[14] Le, M. T., Porcelli, I., Weight, C. M., Gaskin, D. J. H., Carding, S. R., & Van Vliet, A. H. M. (2012). Acid-shock of *Campylobacter jejuni* induces flagellar gene expression and host cell invasion. *European Journal of Microbiology and Immunology*, *2*(1), 12–19. https://doi.org/10.1556/EuJMI.2.2012.1.3

[15] Lynch, C. T., Lynch, H., Burke, S., Hawkins, K., Buttimer, C., Mc Carthy, C., Egan, J., Whyte, P., Bolton, D., Coffey, A., & Lucey, B. (2020). Antimicrobial Resistance Determinants Circulating among Thermophilic Campylobacter Isolates Recovered from Broilers in Ireland Over a One-Year Period. *Antibiotics*, *9*(6), 308. https://doi.org/10.3390/antibiotics9060308

[16] Madden, R. H., Moran, L., Scates, P., Mcbride, J., & Kelly, C. (2011). Prevalence of Campylobacter and Salmonella in Raw Chicken on Retail Sale in the Republic of Ireland. *Journal of Food Protection*, *74*(11), 1922–1926. https://doi.org/10.4315/0362-028X.JFP-11-104

[17] Miller, W. G., Chapman, M. H., Yee, E., Revez, J., Bono, J. L., & Rossi, M. (2017). Complete Genome Sequence of the Hippuricase-Positive Campylobacter avium Type Strain LMG 24591. *Genome Announcements*, *5*(43), e01221-17. https://doi.org/10.1128/genomeA.01221-17

[18] Mushi, M., Paterno, L., Tappe, D., Pendo, A., Seni, J., Moremi, N., Mirambo, M., & Mshana, S. (2014). Evaluation of detection methods for Campylobacter infections among under-fives in Mwanza City, Tanzania. *Pan African Medical Journal*, *19*. https://doi.org/10.11604/pamj.2014.19.392.4242

[19] Oyarzabal, O. A., Williams, A., Zhou, P., & Samadpour, M. (2013). Improved protocol for isolation of Campylobacter spp. From retail broiler meat and use of pulsed field gel electrophoresis for the typing of isolates. *Journal of Microbiological Methods*, *95*(1), 76–83. https://doi.org/10.1016/j.mimet.2013.03.008

[20] Paintsil, E. K., Ofori, L. A., Akenten, C. W., Fosu, D., Ofori, S., Lamshöft, M., May, J., Danso, K. O., Krumkamp, R., & Dekker, D. (2021). Antimicrobial Usage in Commercial and Domestic Poultry Farming in Two Communities in the Ashanti Region of Ghana. *Antibiotics*, *10*(7), 800. https://doi.org/10.3390/antibiotics10070800

[21] Pergola, S., Franciosini, M. P., Comitini, F., Ciani, M., De Luca, S., Bellucci, S., Menchetti, L., & Casagrande Proietti, P. (2017). Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. *Journal of Applied Microbiology*, *122*(5), 1348–1356. https://doi.org/10.1111/jam.13419

[22] Rodrigues, C. S., Armendaris, P. M., De Sá, C. V. G. C., Haddad, J. P. A., & De Melo, C. B. (2021). Prevalence of Campylobacter spp. In Chicken Carcasses in Slaughterhouses from South of Brazil. *Current Microbiology*, *78*(6), 2242–2250. https://doi.org/10.1007/s00284-021-02478-w

[23] Sathiamoorthi, T., Joseph Sahayarayan, J. and Arivoli, A. (2016). PREVALENCE OF MULTI DRUG RESISTANCE CAMPYLOBACTER JEJUNI FROM RAW CHICKENMEAT IN ERODE RETAIL SHOP. *International Journal of Current Research*, *8*(9), 38612–38614.

[24] Serwecińska, L. (2020). Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water*, *12*(12), 3313. https://doi.org/10.3390/w12123313

[25] Sheppard, S. K., & Maiden, M. C. J. (2015). The Evolution of *Campylobacter jejuni* and *Campylobacter coli*. *Cold Spring Harbor Perspectives in Biology*, *7*(8), a018119. https://doi.org/10.1101/cshperspect.a018119

[26] Szczepanska, B., Andrzejewska, M., Spica, D., & Klawe, J. J. (2017). Prevalence and antimicrobial resistance of Campylobacter jejuni and Campylobacter coli isolated from children and environmental sources in urban and suburban areas. *BMC Microbiology*, *17*(1), 80. https://doi.org/10.1186/s12866-017-0991-9

[27] Szczepańska, B., Kamiński, P., Andrzejewska, M., Śpica, D., Kartanas, E., Ulrich, W., Jerzak, L., Kasprzak, M., Bocheński, M., & Klawe, J. J. (2015). Prevalence, Virulence, and Antimicrobial Resistance of *Campylobacter jejuni* and *Campylobacter coli* in White Stork *Ciconia ciconia* in Poland. *Foodborne Pathogens and Disease*, *12*(1), 24–31. https://doi.org/10.1089/fpd.2014.1793

[28] Szosland-Fałtyn, A., Bartodziejska, B., Królasik, J., Paziak-Domańska, B., Korsak, D., & Chmiela, M. (2018). The Prevalence of *Campylobacter* spp. In Polish Poultry Meat. *Polish Journal of Microbiology*, *67*(1), 117–120. https://doi.org/10.5604/01.3001.0011.6152

[29] Tang, M., Zhou, Q., Zhang, X., Zhou, S., Zhang, J., Tang, X., Lu, J., & Gao, Y. (2020). Antibiotic Resistance Profiles and Molecular Mechanisms of Campylobacter from Chicken and Pig in China. *Frontiers in Microbiology*, *11*, 592496. https://doi.org/10.3389/fmicb.2020.592496

[30] Thomrongsuwannakij, T., Blackall, P. J., & Chansiripornchai, N. (2017). A Study on *Campylobacter jejuni* and *Campylobacter coli* through Commercial Broiler Production Chains in Thailand: Antimicrobial Resistance, the Characterization of DNA Gyrase Subunit A Mutation, and Genetic Diversity by Flagellin A Gene Restriction Fragment Length Polymorphism. *Avian Diseases*, *61*(2), 186–197. https://doi.org/10.1637/11546-120116-Reg.1

[31] Vandeputte, J., Martel, A., Canessa, S., Van Rysselberghe, N., De Zutter, L., Heyndrickx, M., Haesebrouck, F., Pasmans, F., & Garmyn, A. (2019). Reducing Campylobacter jejuni colonization in broiler chickens by in-feed supplementation with hyperimmune egg yolk antibodies. *Scientific Reports*, *9*(1), 8931. https://doi.org/10.1038/s41598-019-45380-z

[32] Vlieghe, E. R., Jacobs, J. A., Van Esbroeck, M., Koole, O., & Van Gompel, A. (2008). Trends of Norfloxacin and Erythromycin Resistance of *Campylobacter jejuni/Campylobacter coli* Isolates Recovered From International Travelers, 1994 to 2006. *Journal of Travel Medicine*, *15*(6), 419–425. https://doi.org/10.1111/j.1708-8305.2008.00236.x

[33] Walker, L. J., Wallace, R. L., Smith, J. J., Graham, T., Saputra, T., Symes, S., Stylianopoulos, A., Polkinghorne, B. G., Kirk, M. D., & Glass, K. (2019). Prevalence of Campylobacter coli and Campylobacter jejuni in Retail Chicken, Beef, Lamb, and Pork Products in Three Australian States. *Journal of Food Protection*, *82*(12), 2126–2134. https://doi.org/10.4315/0362-028X.JFP-19-146

[34] Wieczorek, K., & Osek, J. (2013). Antimicrobial Resistance Mechanisms among *Campylobacter*. *BioMed Research International*, *2013*, 1–12, https://doi.org/10.1155/2013/340605

[35] Yang, Y., Feye, K. M., Shi, Z., Pavlidis, H. O., Kogut, M., J. Ashworth, A., & Ricke, S. C. (2019). A Historical Review on Antibiotic Resistance of Foodborne Campylobacter. *Frontiers in Microbiology*, *10*, 1509. https://doi.org/10.3389/fmicb.2019.01509