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High Prevalence of Resistance to Fluoroquinolones and Tetracycline *Campylobacter* Spp. Isolated from Poultry in Poland

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Campylobacter spp. is a major cause of foodborne diseases in humans, particularly when transmitted by the handling or consumption of undercooked poultry meat. Most *Campylobacter* infections are self-limiting, but antimicrobial treatment (*e.g*., fluoroquinolones and macrolides) is necessary in severe or prolonged cases. The indiscriminate use of these drugs, both in clinical medicine and animal production, has a major impact on public health. The aim of the present study was to identify *Campylobacter* strains, isolated from turkey and broilers, using both PCR and the matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) methods to reveal the accuracy of identification, as well to evaluate the antimicrobial and genetic resistance of the investigated strains. MALDI-TOF and PCR methods were used to show differences, if any, in the specificity of that test. In this study, MALDI-TOF mass spectrometry gave the same results as multiplex PCR, in all cases. The highest rate of resistance (*i.e*., 100% of turkey and broiler strains) was detected against ciprofloxacin, whereas 58.1% of turkey and 78.6% of broiler strains were resistant to tetracycline. Multidrug-resistant isolates were not found in the study. All ciprofloxacin-resistant strains had a mutation in the *gyrA* gene, at the Thr-86 position. The presence of the *tetO* gene was found in 71% of turkey and in 100% of broiler strains. All resistant to tetracycline strains included *tetO* gene. Additionally, in five turkey and three broiler strains, susceptible to tetracycline, *tetO* gene was present. These results indicate the high prevalence of *Campylobacter* strains, which are phenotypically and genetically resistant to fluoroquinolones and tetracycline.

Keywords: *Campylobacter*, antimicrobials, MALDI-TOF MS, PCR

Introduction

Campylobacter spp. is a major cause of foodborne illness worldwide.[1] According to EFSA Reports presented in the last few years, the prevalence of *Campylobacter* spp. in humans has an increasing prominence.[2] Among *Campylobacter* species, *C. jejuni* and *C. coli* are the most common bacterial cause of human gastroenteritis.[2] Poultry and poultry products are considered to be the main reservoir and source of transmission of *Campylobacter*, to humans.[2,3] Campylobacteriosis is usually a self-limiting infection that typically does not require any antimicrobial therapy; children, the elderly, and patients who are immunocompromised are at a higher risk for severe diarrhea that requires hospitalization. *C. jejuni* infections occasionally lead to complications, such as bacteremia and postinfection reactive arthritis or Guillain–Barré Syndrome.[4,5]

For many decades, *Campylobacter* spp. has been identified using serological and biochemical methods, which are time consuming and unreliable. The PCR method has more complementary value, but it is also time consuming. In more recent years, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been developed. This method was used as a suitable technique for the specification of *Campylobacter* strains, to their genera, based on ''species-identifying,'' biomarker ions.[6] Comparing with genetic approach of microbial identification, MALDI-TOF Biotyper is found to be less demanding in laboratory probe preparation, definitely cheaper, and time-saving method. Based on the obtained ''protein fingerprint'' results MALDI-TOF Biotyper software allows direct construction of dendrograms showing phylogenetic relation of strains. A further study on full-spectral MALDI-TOF MS analysis allowed the establishment of a reference

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database of selected *Campylobacter* strains, based on a list of up to 70 peaks, obtained from a set of mass spectra. This was a starting point, to the confirmation of a main spectrum, containing information about mean peak masses, mean peak intensities, and peak frequency.[7]

An increasing trend within the antibiotic resistance of *Campylobacter* spp. has been observed, in the last few years. C*ampylobacter* spp., as a zoonotic bacteria, is exposed to antibiotics, used in both animal production and clinical medicine. The drug of choice for the treatment of human campylobacteriosis is erythromycin (*i.e*., a macrolide), but ciprofloxacin (*i.e*., a fluoroquinolone) is also frequently applied, because of its broad antibacterial spectrum. Alternatively, with a systemic infection of *Campylobacter* spp., tetracycline has also been applied.[8] In poultry industry, these antimicrobials are used for the treatment of respiratory or gastrointestinal disorders. Furthermore, it is well known that ciprofloxacin-resistant *C. jejuni* may colonize poultry intestinal tracts better than the isogenic susceptible *C. jejuni*, even in the absence of fluoroquinolone selection pressure.[9]

Regarding molecular mechanism explaining antimicrobial resistance of *Campylobacter* spp. to different groups of antimicrobials, several genes were identified. The main resistance mechanism of *Campylobacter* spp. to fluoroquinolones is a point mutation in the *gyrA* gene (most commonly leading to Thr-86-Ile), which encodes part of the DNA gyrase, in the quinolone resistance-determining region.[10] Resistance to tetracycline is primarily mediated by a plasmid-encoded *tetO* gene.[11,12] High-level resistant *Campylobacter* spp. to erythromycin, is mediated by a mutation at position A2074C or at A2075G, in domain V of the 23S rRNA.[13,14] Multidrug efflux pumps, such as CmeABC and CmeDEF, are also involved in the resistance of *Campylobacter*, to a broad spectrum of antimicrobials. It has been found that CmeABC functions synergistically, with target mutations, in conferring and maintaining high-level resistance to fluoroquinolones and macrolides.[15,16]

The aim of this study was to identify *Campylobacter* spp., isolated from turkeys and broilers, using two selected methods (PCR and MALDI-TOF) and to evaluate the antibiotic resistance of investigated strains. Regarding the development of microorganism identification methods, MALDI-TOF Biotyper is more recent than PCR method, so it was important to check its effectiveness for speciation of thermophilic *Campylobacter* isolated from turkeys and broilers in Poland. Moreover, the aim of our research was to analyze the prevalence of point mutation or the presence of genes, responsible for antimicrobial resistance to fluoroquinolones, erythromycin and tetracycline, and the prevalence of multidrug efflux pump genes in the population of *Campylobacter* spp. strains.

Materials and Methods

Sample preparation for Campylobacter *spp. identification*

Fresh stool samples were collected, in 2015, in Lower Silesia region, from five commercial turkey farms (MK, MS, Z, O, and Ko) and two broiler farms (1 and 3). The strains marked as MK1, 2, 4–6 (five isolates), and MK9-11 (three isolates) were collected from two separate turkey farms, which were closely located, less than 1 km. Therefore, they are marked the same name—MK. On each farm, few turkey or broiler houses were located (from 3 to 7). From each turkey or broiler house only one strain of *Campylobacter* spp. was isolated. In general, 31 of turkey and 14 of broiler houses (flocks) were sampled (Table 1). Material was taken with sterile swabs and placed into a Bolton Selective Enrichment Broth (Oxoid, Basingstoke, Hampshire, UK). Swabs were then transported to the laboratory, at a temperature of 4°C (±2°C), not exceeding a shipment time of 6 hours.

The collected samples were swabbed onto Columbia Blood Agar plates (Oxoid) with a Campylobacter Selective Supplement, SR0069 (Oxoid). Agar plates were incubated at

Table 1. Species Identification of *Campylobacter* Isolates ($n = 45$) Using the Matrix-Assisted, Laser Desorption Ionization Biotyper and Multiplex PCR

	Identification		
Source and isolate number	*MALDI-TOF Biotyper*	*Score*	*Multiplex PCR*
Reference strains			
Campylobacter coli ATCC 33559	*C. coli*	2.41	*C. coli*
Campylobacter jejuni ATCC 33560	*C. jejuni*	2.29	*C. jejuni*
Turkey strains			
MK1, MK2, MK4, MK5, MK6, MK9, MK10, MK11	*C. jejuni* ($n = 30$)	2.12–2.42	All listed strains confirmed as *C. jejuni*
MS1, MS2, MS3, MS4, MS8, MS10			
Z1, Z2, Z5, Z7			
O1, O2, O3, O4, O5, O6			
Ko4, Ko5, Ko6, Ko7, Ko8, Ko9			
Z3	*C. coli* ($n = 1$)	2.03	Confirmed as *C. coli*
Broiler strains			
1.3, 1.4, 1.5, 1.6, 1.8, 1.9, 1.10	*C. jejuni* ($n = 11$)	2.10–2.43	All listed strains confirmed as *C. jejuni*
3.3, 3.4, 3.5, 3.6			
3.2, 3.7, 3.8	*C. coli* ($n = 3$)	2.20–2.30	All listed strains confirmed as *C. coli*

MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry.

42°C, for 48 hours, in microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2). One typical *Campylobacter* colony (smooth, flat, and colorlessly translucent to gray) was selected for further biochemical investigation; positive motility, and curved shape, Gram-negative and an oxidase- and catalase-positive test. All positive isolates were stored frozen, in glycerol broth, at –70°C, for further molecular identification and antimicrobial resistance.

MALDI-TOF MS analysis for Campylobacter *spp. identification*

Two to five colonies of actively growing cultures, incubated for 24 hours on a blood agar at 42°C, were suspended in 300 μl of double-distilled water. Then, 900 μl of absolute ethanol was added. The sample was centrifuged twice (at 13,000 *g*, for 3 minutes) and the sediment was dried at room temperature. Lysates were prepared by adding 50 μl of 70% formic acid to the bacterial pellet, mixing thoroughly, adding 50 μl of acetonitrile, before mixing the sample again. Following further centrifugation (at 13,000 *g*, for 2 minutes) the supernatant was transferred to a fresh tube and 1 μl of the bacterial protein lysate was applied to a 384 ground steel MALDI target plate (Bruker Daltonics, Bremen, Germany) and air dried at room temperature. Next, the sample was overlaid with 1 μl of α-cyano-4-hydroxycinnamic acid matrix solution (Bruker Daltonics) and air dried, again. Measurements were performed using a Bruker Daltonics UltrafleXtreme spectrometer. Spectra were recorded in the positive linear mode, for a mass range of 2,000–20,000 Da (laser frequency 200 Hz; ion source voltage one, 25 kV; ion source voltage two, 23.5 kV; lens voltage, 6.0 kV). Each spectrum was obtained by averaging 1,500 laser shots, acquired from three spot positions, under the control of FlexControl software 3.1 (Bruker Daltonics). Spectra were externally calibrated using an *Escherichia coli* DH5-alpha standard (Bruker Daltonics). The calibrant consisted of six ribosomal proteins from *E. coli*, with added RNAse A and myoglobin, to cover a range of 3,637.8–16,952.3 Da. Biotyper 3.1 software (Bruker Daltonics) and a database containing 4,613 entries was used for identification. According to the manufacturer, the following score values were used; less than 1.7—identification not reliable, 1.7–2.0—probable genus identification, 2.0–2.3—secure genus identification and probable species identification, and more than 2.3—highly probable species identification.

For dendrogram preparation, principal component analysis (PCA) of sets of spectra was used. This statistical analysis provides information on the hetero/homogeneity of a dataset. PCA was managed by an external MATLAB software tool that was integrated into the MALDI Biotyper.

DNA extraction

Genetic material was isolated using Genomic Mini (A&A Biotechnology, Gdańsk, Poland) in accordance with the manufacturer's recommendations. DNA was quantified spectrophotometrically (BioPhotometer, Eppendorf, Hamburg, Germany) and stored at –20°C.

Multiplex PCR

A single PCR method was used to identify genus *Campylobacter*, whereas the species level of *C. jejuni* or *C. coli* was determined using multiplex PCR. Primer sequences, specific for the simultaneous amplification of the *mapA* gene (*C. jejuni*) and the *ceuE* gene (*C. coli*) are listed in Table 2.[17–19] Protocol for single and multiplex PCR was described by Denis *et al.*[20] The final volume of reaction mixture was 25 μl and contained 1 U of Dream Taq DNA Polymerase (5 U/μl) (Thermo Fisher Scientific, Tewksbury, MA). The PCR program for single and multiplex PCR was as follows: 10 minutes at 95°C, 35 cycles consisted of 30 seconds at 95°C, 1.5 minutes at 59°C, 1 minute at 72°C, and final extension step of 10 minutes at 72°C. Strains used as positive controls were *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559. Products obtained by amplification were separated, through the electrophoresis method, in a 2% agarose gel. DNA bands were stained with a Midori Green DNA Stain (Nippon Genetics Europe GmbH, Dueren, Germany) and visualized with an UV transilluminator.

Antimicrobial resistance

Nine antimicrobial agents (azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin, and clindamycin) were tested to show the susceptibility of *Campylobacter* strains. Some of these antimicrobials, especially fluoroquinolones and macrolides, are the drugs of choice in the treatment of *Campylobacter* infection in humans. The minimal inhibitory concentration (MIC) of each drug was determined by broth microdilution, using a Sensititre™ Campylobacter MIC Plate (TREK Diagnostic System, West Sussex, UK) according to the manufacturer's instructions. Colonies were harvested on a Columbia Blood Agar Base (Oxoid) supplemented with 7% of sheep blood, incubated in microaerophilic conditions, for 48 hours, at 42°C, and then seeded in a Mueller-Hinton Broth Supplement with blood, and added to microtiter

Table 2. Primer Sequences Used for *Campylobacter* Species Identification

Species	*Gene*	*Sequence (5′→3′)*	*Amplicon (bp)*	*References*
C. spp.	*16S rRNA*	AGTCTTGGCAGTAATGCACCTAACG ATATGCCATTGTAGCACGTGTGTCG	408	Wangroongsarb *et al.*[19]
C. jejuni	*mapA*	CTATTTTATTTTTGAGTGCTTGTG GCTTTATTTGCCATTTGTTTTATTA	589	Stucki *et al.*[18]
C. coli	*ceuE*	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTATTTGTAGCAGCG	462	Gonzalez *et al.*[17]

Table 3. List of Primers Used for the Molecular Detection of Antimicrobial Resistance

Gene	Primers	Sequence (5′→3′)	Amplicon (bp)	References
Ciprofloxacin				
gyrA	cjgyrM1	AAATCAGCCCGTATAGTGGGTG CTGTTATAGGTCGTTATCA CCCACACATGGAGGT	179	Wardak *et al.*[24]
	cjgyrA2	TCAGTATAACGCATCGCAGC		
Tetracycline				
tetO	DMT 1	GGCGTTTTGTTTATGTGCG	559	Gibreel *et al.*[12]
	DMT 2	ATGGACAACCCGACAGAAGC		
Erythromycin				
23S rRNA	F2-campy-23S	AATTGATGGGGTTAGCATTAGC	316	El-Adawy *et al.*[25]
	R2-campy-23S	CAACAATGGCTCATATACAACTGG		
Efflux pumps				
cmeB	EP 1	TCCTAGCAGCACAATATG	241	Obeng *et al.*[26]
	EP 2	AGCTTCGATAGCTGCATC		

plates. Plates were incubated for 24 hours, in the same conditions and then screened. Assays were repeated twice, each in duplicate, to confirm the reproducibility of the MIC data. Interpretation of the obtained results was performed to the interpretative standard, as recommended by the Clinical and Laboratory Standards Institute.[21,22] The reference strains of *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were used as test controls.

Molecular methods for detection of antimicrobial resistance

Ciprofloxacin resistance. To detect a point mutation in the *gyrA* gene, which correlates to fluoroquinolone resistance, PCR-RFLP was used. The sequences of the primers used in this study are presented in Table 3. PCR amplification of the *gyrA* gene of *C. jejuni* and *C. coli* was performed, using primers and the reaction condition described by Alonso *et al.*[23] and Wardak *et al.*[24] A PCR product of size 179-bp was digested by restriction enzyme *Rsa*I (Thermo Fisher Scientific, Waltham, MA) with an effect at 10 U/μl.

Tetracycline resistance. The conserved primers, DMT 1 and DMT 2, were used to amplify a 559-bp PCR product of the *tetO* gene that is associated with resistance to tetracycline, in *Campylobacter* isolates. PCR was done as previously described.[12]

Erythromycin resistance. All isolates were tested for the presence of mutation in the 23S rRNA gene, using PCR-RFLP, according to the protocols described by El-Adawy *et al.*[25] The amplified PCR products of 316-bp were digested with a *Bsa*I restriction enzyme (Thermo Fisher Scientific, Waltham, MA).

Efflux pumps. All *Campylobacter* spp. were tested for the presence of the *cmeB* gene. PCR was carried out, using the amplification protocols described by Obeng *et al.*[26] The lengths of various amplicons, obtained in each PCR, were determined by comparing them with a GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA).

Results

Bacterial identification

In total, 45 *Campylobacter* strains were isolated from turkeys (31; 68.9%) and broilers (14; 31.1%). Among all investigated strains, 41 (91.1%) were identified as *C. jejuni*, whereas only 4 (8.9%) as *C. coli.* The number of *C. jejuni* and *C. coli* isolated from turkeys were as follows: 30 (96.8%) and only 1 (3.2%), whereas the number of *C. jejuni* and *C. coli* obtained from broilers were, 11 (78.6%) and 3 (21.4%), respectively (Table 1).

All isolates from turkeys and broilers were also identified, with the MALDI Biotyper method. The results presented in Table 3 confirm multiplex PCR data, recognizing 41 strains as *C. jejuni* and 4 strains as *C. coli*. Based on score values over 2.00, obtained for all 45 samples, species identification was deemed as complete.

Furthermore, based on MALDI Biotyper spectra, a PCA Dendrogram of all the studied strains was obtained. As presented in Fig. 1, a cluster separation of *C. coli* strains, from the *C. jejuni* strains, with a high distance level, was observed. Interestingly, isolate 3.2, identified as *C. coli*, was positioned in a branch together with *C. jejuni*, as with other strains isolated from turkeys.

Antimicrobial resistance

The results of the resistance of *Campylobacter* strains, to selected antimicrobials, and the rate of resistance to each antimicrobial agent are shown in Table 4. All investigated strains, isolated from turkeys and broilers, were susceptible to azithromycin, erythromycin, gentamicin, florfenicol, telithromycin, and clindamycin, whereas all strains were resistant to ciprofloxacin. Also, 92.9% and 78.6% of broiler strains were resistant to nalidixic acid and tetracycline, whereas 83.9% and 58.1% of turkey isolates were resistant to nalidixic acid and tetracycline, respectively. Similar resistance patterns were observed between *C. jejuni* and *C. coli* isolated from turkeys, whereas all *C. coli* isolated from broilers ($n=3$) were susceptible to tetracycline, in comparison to *C. jejuni*, where all strains were resistant to this antibiotic. Multidrug resistance to three or more classes of antimicrobial agents was not found between the investigated strains.

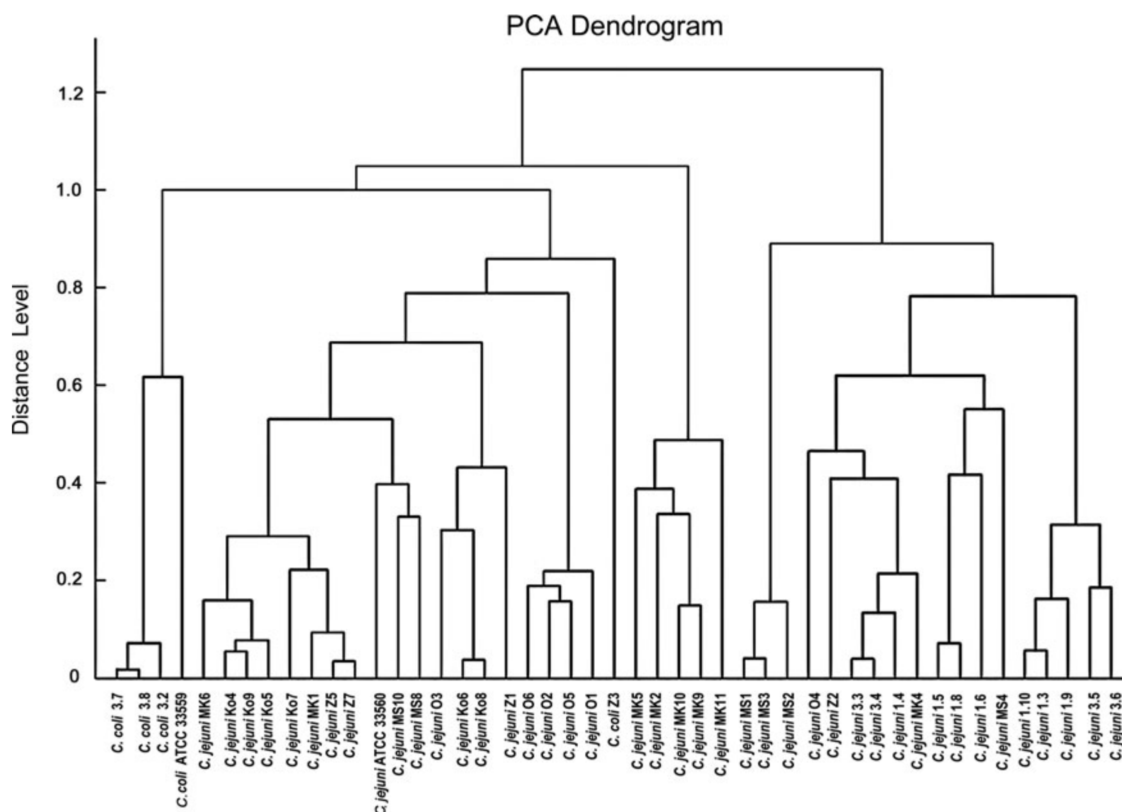

FIG. 1. Principal component analysis dendrogram of *Campylobacter* isolates and reference strains.

Molecular detection of antimicrobial resistance

All phenotypically, ciprofloxacin-resistant strains had a mutation in the *gyrA* gene, at the Thr-86 position (Table 5). The presence of the *tetO* gene was found in 21 (70%) of *C. jejuni* and in 1 (100%) of *C. coli* strains isolated from turkeys. Among the *C. jejuni* obtained from broilers, all tetracycline-resistant strains (11/100%) possessed the *tetO* gene. Notably, *C. coli* isolated from broilers, which were phenotypically tetracycline-susceptible, were found to encode the *tetO* and *cmeB* genes, as well. Comparing the presence of the *cmeB* gene between turkey and broiler isolates, 4 (13.3%) of the *C. jejuni* isolated from turkeys had a *cmeB* gene, whereas among broiler strains, 3 of the *C. coli* (100%) were found to encode the *cmeB* gene. All investigated strains possessing a *cmeB* gene, were obtained from one farm, in the case of turkey and broiler isolates, separately. The results of PCR-RFLP showed that no strain had a restriction site for *Bsa*I, demonstrating the absence of mutation in all of the erythromycin-susceptible strains.

Discussion

The development of MALDI-TOF MS technology, for bacterial strain identification, is a simple, low-cost, and rapid method. In this study, MALDI-TOF MS produced correct identifications for all investigated *Campylobacter* strains, in comparison with a PCR method. The accuracy of MALDI-TOF MS identification has also been proved by other authors.[7,27,28] Bessède *et al.*[27] analyzed over one thousand strains and the results were compared with the gold standard of *Campylobacter* species identification, using real-time PCR and the sequencing of a 444-base-pair fragment of the *gyrA* gene. The accuracy of MALDI-TOF MS reached 100%, compared with the gold standard, for all of the *Campylobacter* species, except *C. jejuni* (99.4%). For the confirmation and comparison purposes, the classifications achieved through these techniques were compared with 16S rRNA sequence-based phylogenetic analysis.

A recent study focused on employing MALDI-TOF MS, together with Raman and FT-IR spectroscopies, combined with a multivariate statistical analysis, for the differentiation of *Campylobacter*, down to the subspecies level.[29] The classifications achieved, through these techniques, were compared with a 16S rRNA sequence-based phylogenetic analysis, for confirmation and evaluation purposes. Results demonstrated that such metabolomic approaches, combined with molecular biology techniques, may provide critical information and knowledge, related to risk factors, virulence, and understanding of the distribution and transmission routes associated with different strains of foodborne *Campylobacter* spp.

Clustering analysis, using PCA dendrograms, generated by MALDI Biotyper mass spectra, for bacterial isolates, allows specification of the phylogenetic affiliation of the individual isolates, during population-based study.[30]

Thermophilic *Campylobacter* are zoonotic pathogens; therefore, the development of antimicrobial resistance, in these bacteria, is a matter of great concern. There is a hypothesis that unregulated use of antimicrobial agents, in food-animal production, has led to the emergence and spread of antibiotic resistance, among *Campylobacter* spp. The prevalence of resistant strains is very low, in

Table 4. Antimicrobial Resistance of *C. jejuni* and *C. coli*, Isolated from Turkeys and Broilers

Species	Antimicrobials (MIC μg/ml)	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64	MIC50	MIC90	R**%
Turkey strains	AZI	7	23	1											>0.03	>0.03	0
C. jejuni (*n* = 30)	CIP									2	19	9	0	1	>8	>16	100.0
C. coli (*n* = 1)	ERY		2	2	5	20	2								>0.25	>0.25	0
	GEN				1	23	6		1						>0.25	>0.5	0
	TET				7	6								18 (1*)	>64	>64	58.1
	FFN					1	16	11	3						>0.5	>1	0
	NAL											5	2 (1*)	24	>64	>64	83.9
	TEL		1	2	4	6	14	4							>0.5	>1	0
	CLI		18	9	4										>0.03	>0.12	0
Broiler strains	AZI		11	3											>0.03	>0.06	0
C. jejuni (*n* = 11)	CIP									1	8	5			>8	>16	100.0
C. coli (*n* = 3)	ERY			1	6	4	3								>0.12	>0.5	0
	GEN				2	7	3	2							>0.25	>1	0
	TET						2*	1*					1	10	>64	>64	78.6
	FFN						4	7	3						>1	>2	0
	NAL											1	3 (1*)	10 (2*)	>64	>64	92.9
	TEL			2	4	2	3	3							>0.25	>1	0
	CLI		4	6	4										>0.06	>0.12	0

Grey shaded area indicates the resistant strains and *white shaded area* indicates susceptible strains. $MIC_{50} = (n \times 0.5)$; $MIC_{90} = (n \times 0.9)$.
*The number of *Campylobacter coli* strains included in the total amount of sensitive/resistant isolates.
**R, resistance rate.
AZI, azithromycin (0.015–64 μg/ml); CIP, ciprofloxacin (0.015–64 μg/ml); ERY, erythromycin (0.03–64 μg/ml); GEN, gentamicin (0.12–32 μg/ml); TET, tetracycline (0.06–64 μg/ml); FFN, florfenicol (0.03–64 μg/ml); NAL, nalidixic acid (4–64 μg/ml); TEL, telithromycin (0.015–8 μg/ml); CLI, clindamicin (0.03–16 μg/ml); MIC, minimal inhibitory concentration.

TABLE 5. PRESENCE OF RESISTANT GENES/MUTATIONS IN *C. JEJUNI* AND *C. COLI*, ISOLATED FROM TURKEYS AND BROILERS

	Turkey isolates (n = *31*)		*Broiler isolates* (n = *14*)	
Resistant genes/mutations	C. jejuni *(n = 30)*	C. coli *(n = 1)*	C. jejuni *(n = 11)*	C. coli *(n = 3)*
Mutation in *gyrA*	30 (100.0)	1 (100.0)	11 (100.0)	3 (100.0)
tetO	21 (70.0)	1 (100.0)	11 (100.0)	3 (100.0)
Mutation in 23S rRNA	0	0	0	0
cmeB	4 (13.3)	0	0	3 (100.0)

countries where the use of antibiotics in poultry industry is uncommon.[31] Norström *et al.* indicated a very low resistance rate to oxytetracycline (1.3%), whereas resistance to quinolones was not observed. Other authors, from Italy, revealed a high level of resistance to ciprofloxacin (62.8%), tetracycline (55.9%), and nalidixic acid (55.2%).[32] High levels of the resistance of *Campylobacter* isolates to fluoroquinolones have also been found in Latvia, Lithuania, Spain, and Iran.[33–35] Zhao *et al.*[36] have shown 13 multidrug resistance profiles of *C. jejuni* and *C. coli*, isolated from humans, retail meats, and cecal samples of food production animals (*e.g.*, chicken and turkey). Strains were resistant to tetracycline, gentamicin, azithromycin, clindamycin, telithromycin, erythromycin, and ciprofloxacin. The highest rate of resistance was observed for tetracycline, 94.7% of all isolates. A similar, high incidence of tetracycline resistance of *C. jejuni* and *C. coli,* isolated from broiler carcasses, was reported in Brazil and the United States, 75% and over 96%, respectively.[37,38] The present study provides the highest rate of resistance to fluoroquinolones (*i.e.*, ciprofloxacin and nalidixic acid), and to tetracycline. Both of these antimicrobial groups are often used in veterinary and human medicine, especially regarding enteric infections. All strains isolated from turkeys and broilers were resistant to ciprofloxacin, whereas 83.9% of turkey isolates and 92.9% of broiler isolates were resistant to nalidixic acid. The rate of resistance to fluoroquinolones and tetracycline in poultry has increased over the last dozen years, in Poland. Woźniak[39,40] reported that the rate of resistance of *Campylobacter*, isolated from broilers, to tetracycline, rose from 0% in 1994–1996 to 18.7% in 2005–2008, and to ciprofloxacin, rose from 43.6% in 1994–1996 to 85.4% in 2005–2008.

In the current study, all of the *Campylobacter* isolates were susceptible to azithromycin, erythromycin, gentamicin, florfenicol, telithromycin, and clindamycin, probably because of the rare use of these antimicrobials in Polish poultry production. Other authors[25,32,37] have revealed similar results, where all isolates were susceptible to gentamicin and less than 5% of strains were resistant to chloramphenicol. In general, the rate of resistance to erythromycin seems to be low,[26,33,35,37,41] which is in agreement with the results of this study.

Our study revealed a high correlation between phenotypic resistance to a given drug tested and the presence of the gene/mutation expected to confer resistance to that drug, which is in agreement with previous research.[24,25,34,42] With regard to fluoroquinolones, mutation Thr86Ile is the most prevalent in isolates, among other described mutations (*i.e.*, Thr86Ala, Thr86Lys, Ala87Pro, Asp90His, and Asp90Tyr).[43,44] In this study, in relation to ciprofloxacin and nalidixic acid, there was a 100% correlation to the presence of a Thr86Ile mutation and the observed quinolone resistance phenotype. Our findings are similar to El-Adawy *et al.*[25] who revealed the same mutation in all ciprofloxacin-resistant *Campylobacter* strains.

Resistance to tetracycline is mostly associated with the presence of the *tetO* resistant gene. In this study, all 18 tetracycline-resistant strains isolated from turkeys and 11 tetracycline-resistant strains isolated from broilers carried the *tetO* gene, whereas 5 tetracycline-susceptible *C. jejuni* isolated from turkeys and 3 tetracycline-susceptible *C. coli* obtained from broilers were also carrying the *tetO* gene. These findings indicate that the *tetO* gene, in these *Campylobacter* isolates, may have been inactive or were not being expressed. Obeng *et al.*[26] found 3.8% of tetracycline-susceptible *C. jejuni*, from chickens, with the presence of the *tetO* gene. Pratt and Korolik[45] revealed that the *tetO* gene was found in all tetracycline-resistant *Campylobacter*, of human and poultry origin, in Australia, whereas PCR amplification was successful in detecting the *tetO* gene in 97.8% of investigated strains.

The main mechanisms conferring resistance against macrolides, in *Campylobacter*, is transition A2075G.[46] This study found no strains with mutation A2075G, which is in agreement with their susceptibility to this antimicrobial and with other studies.[25] Pérez-Boto *et al.*[34] revealed only 1 *C. coli* strain resistant to erythromycin, where the mutation at A2075G (23S r DNA) was responsible for macrolide resistance. Wirz *et al.*[47] detected the A2075G transition, only in 3.1% of *C. coli* isolates from broilers.

In this study, the *cmeB* gene was carried by a low number (13.3%) of *C. jejuni* strains from turkeys, whereas the same was found in all (100%) *C. coli* isolated from broilers. The occurrence of this gene was significantly higher in *C. coli* than in *C. jejuni*, which is consistent with other studies.[26,48]

Conclusions

Campylobacter identification, using the MALDI-TOF MS method, considering the speed with which reliable identification can be obtained, is well suited for large-scale research and diagnostic analyses. Additionally, this technology allows the analysis of the phylogenetic origin of investigated strains.

In this study, *Campylobacter* from poultry production, showed resistance to a relatively narrow range of antimicrobials. The significant usage of fluoroquinolones and tetracycline in poultry production, in our country, has led, as shown in our study, to the emergence of quinolone and tetracycline-resistant *Campylobacter* strains. Monitoring of antimicrobial resistance for *Campylobacter* and the appropriate use of antimicrobials in animal-food

production are essential, for decreasing drug resistance in bacterial pathogens.

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No competing financial interests exist.

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