Virulence and antibiotic resistance genes in *Campylobacter* spp. in the Czech Republic

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ABSTRACT

Objective: Thermotolerant species of the genus *Campylobacter* are the important agents causing human foodborne infections throughout the world. The aims of this study were to evaluate the presence of nine putative virulence genes in *Campylobacter* spp. isolated from patients and from foods (poultry meat, pork liver), to determine the resistance of *Campylobacter* isolates to eight antibiotic agents and to detect four resistance genes.
Matherial and methods: The presence of the virulence genes *cdtA, cdtB, cdtC, virB11, ciaB, wlaN, iam, dnaJ* and *racR* was detected by polymerase chain reaction (PCR) in 94 *Campylobacter* spp. isolates from humans and 123 campylobacters from foods. The phenotypic resistance to selected antimicrobial agents was tested with microdilution method in 82 human isolates and 91 food isolates. The isolates with antibiograms were tested for the presence of bla_{OXA-61}, *tet(O)*, *aph-3-1* and *cmeB* genes by PCR with specific primers.
Results: In both human and food *C. jejuni* isolates the prevalence of the studied virulence genes, especially *dna*J, *rac*R, *cia*B genes and the toxigenic genes *cdt*A, *cdt*B, *cdt*C, was considerably higher than in *C. coli* isolates. The only exception was the iam gene identified in only *C. coli*. The tested isolates of both *C. jejuni* and *C. coli* were highly resistant to quinolone antibiotics. Additionally, *C. coli* was also more resistant to erythromycin, streptomycin and, in case of isolates from pork liver, to tetracycline. High prevalence rates of genes encoding antibiotic resistance was noted for the bla_{OXA-61} and *tet(O)* genes in both *Campylobacter* species.
Conclusions: The presented study is the first to assess the presence of genes for virulence and resistance to antibiotics in thermotolerant *Campylobacter* spp. isolated from humans and foods in the Czech Republic. The resistance of *Campylobacter* isolates to eight antibiotic agents was also assessed. The prevalence of genes responsible for virulence and resistance is rather varied in thermotolerant *Campylobacter* spp.

KEYWORDS:
***Campylobacter* – foodborne infections – virulence genes – resistance genes**

SOUHRN

Bardoň J., Pudová V., Koláčková I., Karpíšková R., Röderová M., Kolář M.: Geny virulence a rezistence k antibiotikům u *Campylobacter* spp. v České republice

Cíl práce: Termotolerantní druhy bakterií rodu *Campylobacter* jsou významná agens způsobující alimentární infekce člověka na celém světě. Cílem této studie bylo prověřit výskyt devíti předpokládaných genů virulence u kampylobakterů izolovaných od pacientů a z potravin (drůbeží maso, vepřová játra), stanovit rezistenci izolátů k osmi antibiotikům a detekovat u izolátů čtyři geny rezistence k antibiotikům.
Materiál a metody: Výskyt genů virulence *cdtA, cdtB, cdtC, virB11, ciaB, wlaN, iam, dnaJ* a *racR* byl detekován pomocí polymerázové řetězové reakce (PCR) u 94 humánních izolátů a 123 izolátů kampylobakterů z potravin. Fenotypová rezistence izolátů k vybraným antibiotikům byla testována mikrodiluční metodou u 82 humánních izolátů a 91 izolátů z potravin. Izoláty se stanoveným antibiogramem byly testovány na výskyt genů bla_{OXA-61}, *tet(O), aph-3-1* a *cmeB* pomocí specifických primerů metodou PCR.
Výsledky: Prevalence sledovaných genů virulence, zejména *dnaJ, racR, ciaB* a genů toxigenity *cdtA, cdtB, cdtC*, byla podstatně vyšší u izolátů *C. jejuni* od pacientů i z potravin v porovnání s izoláty *C. coli*. Jedinou výjimkou byl gen iam, který byl identifikován pouze u *C. coli*. Testované izoláty *C. jejuni* i *C. coli* byly vysoce rezistentní k chinolonovým antibiotikům. Izoláty *C. coli* byly navíc více rezistentní k erytromycinu a streptomycinu, v případě izolátů z vepřových jater také k tetracyklinu. U obou druhů kampylobakterů byla zaznamenána vysoká prevalence genů rezistence k antibiotikům bla_{OXA-61} a *tet(O)*.
Závěr: Předkládaná studie je první prací, která hodnotí výskyt genů virulence a rezistence u termotolerantních kampylobakterů izolovaných od pacientů a z potravin v České republice. Rovněž je vyhodnocena rezistence izolátů k osmi vybraným antibiotikům. Prevalence genů zodpovědných za virulenci i rezistenci k antibiotikům se u jednotlivých druhů termotolerantních kampylobakterů liší.

KLÍČOVÁ SLOVA:
***Campylobacter* – alimentární infekce – geny virulence – geny rezistence**

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INTRODUCTION

Members of the genus *Campylobacter* are the main pathogens responsible for acute bacterial gastroenteritis in humans throughout the world. Most of the infections are caused by *Campylobacter jejuni* (approximately 90%), followed by *C. coli* (approximately 10%). Foodborne infections due to *C. lari* and *C. upsaliensis* are sporadic [1]. Worldwide, approximately 400 million people a year contract the infection. In developing countries, up to 60% of children younger than 5 years become ill with *Campylobacter*iosis [2].

The infectious dose of *Campylobacter* infections is relatively low and has been estimated at as few as 500 cells [3, 4]. The typical incubation period ranges between 3 to 5 days. The pathogenesis of this human foodborne infection has not been fully elucidated. The survival of *Campylobacter*s in the acid stomach environment upon ingestion can be affected by the buffer capacity of the consumed food. The colonization starts in the jejunum and upper ileum and then spreads to the rest of the ileum and colon. *Campylobacter*s have to overcome the intestinal mucosa, adhere to the epithelial cells and enter them. It is assumed that this is essential for inducing diarrhea [1].

The abilities of *Campylobacter* spp., mainly to adhere to, colonize and invade the intestinal wall and to produce toxins, are encoded by numerous genes responsible for the virulence of their strains. For instance, the genes *flaA*, *cadF*, *racR*, and *dnaJ* are responsible for intestinal adherence and colonization. The genes *virB11*, *ciaB*, *iam* and pldA are responsible for invasiveness [5, 6]. *In vitro* studies have shown that *virB11* gene of *C. jejuni* strains is associated with both adherence and invasion [7]. The gene *wlaN* is responsible for mimicry leading to post-infectious complications in the form of Guillain-Barré syndrome [5]. Another virulence factor that has been proposed to play a role in the pathogenesis is the cytolethal distending toxin (CDT). This cytotoxin consists of three subunits which are encoded by *cdtA*, *cdtB* a *cdtC* genes, and all three subunits are necessary for full activity [8, 6].

The treatment of human *Campylobacter* infections is usually symptomatic; in case of antibiotic therapy, the recommended agents are macrolides or possibly ciprofloxacin [1]. However, many studies in Europe reported high percentages of *Campylobacter* spp. strains isolated from both humans and animals that were completely resistant to quinolones [9]. Tetracyclines have been mentioned as alternative antibiotics for therapy, but they are not used in practice [10].

The targets of quinolone antibiotics are enzymes (namely gyrases/topoisomerases) playing an important role in bacterial synthesis of DNA. The resistance to quinolones in *Campylobacter* spp. is usually caused by mutations in specific regions of target enzymes, but there are some other mechanisms of resistance such as efflux pumps. These systems also play a role in resistance to other antimicrobials, for example macrolides [11]. In *Campylobacter* spp., the CmeABC pump has been described as the main efflux mechanism causing resistance to several classes of antibiotics (beta-lactams, erythromycin, tetracycline) [12]. The resistance of *Campylobacter* spp. to antimicrobial agents is not associated with mutations of target structures or efflux systems only as several resistance genes have been described as well. These are, for example, genes encoding modifying enzymes (*aph*), beta-lactamase (bla_{OXA-61}) or ribosomal protection proteins (*tet(O)*) which are linked with resistance to aminoglycosides, beta-lactams and tetracyclines, respectively [10]. The aims of this study were to evaluate the presence of nine putative virulence genes in *Campylobacter* spp. isolated from patients and from foods (poultry meat, pork liver), to determine the resistance of *Campylobacter* isolates to eight antibiotic agents and to detect four resistance genes.

MATERIALS AND METHODS

Sampling

Between May 2013 and December 2014, samples of fresh chicken, frozen chicken, fresh pork liver and raw cow's milk were regularly collected at 2-month intervals for *Campylobacter* spp. testing. Both poultry (n = 209) and pork liver (n = 103) samples were collected in randomly selected large supermarkets and raw cow's milk samples (n = 110) were obtained from milk vending machines. Over the above period, samples were taken on 10 occasions, with each set comprising approximately 20 poultry, 10 liver and 11 milk samples. All the meat and milk samples to be tested were normally purchased from supermarkets and vending machines in Moravia, the eastern part of the Czech Republic. Thus, commodities directly entering the consumers' food chain were included in the study. Between May 2013 and December 2014, human isolates of *Campylobacter* spp. (n = 235) were obtained from rectal swabs taken from patients with diarrhea. The isolates originated from hospital laboratories (University Hospital Olomouc, University Hospital Brno, St. Anne´s University Hospital Brno, Hospital Prostějov) and laboratories performing tests to detect diarrheal diseases in the above community (Mikrochem Laboratories Olomouc, Laboratories IFCOR-99 Brno). The territory of operation of these laboratories are The Olomouc Region and The South Moravian region, Czech Republic. From each patient, only one isolate was included. Although data on gender, age and primary diagnosis were available for all patients, these were not evaluated in the study.

Detection, isolation and identification of thermophilic *Campylobacter*

Food samples were always delivered to the laboratory on the day of collection. The method for *Campylobacter* spp. detection was based on ISO 10272-1 (qualitative testing) [13]. The samples were in the form of 25 g of skin collected from chicken necks, 25 g of pork liver or 25 ml of raw milk. Culture media as recommended by the above norm were manufactured by Trios and Oxoid. For identification purposes, suspected isolates were inoculated onto blood agar (Trios), and following 48-hour microaerophilic incubation at 42.5 °C, they were identified using the MALDI-TOF MS method (Biotyper Microflex, Bruker). In case of inconclusive results (identification scores < 2), PCR methods with a commercial kit for real-time PCR (Taq Man *Campylobacter* spp. Kit, AB Applied Biosystems) were used [14, 15]. Human isolates of *Campylobacter* spp. had already been identified by the external laboratories that provided them. Prior to further testing, however,

Table 1. The parameters of testing and resistance to the selected antibiotics in *C. jejuni*

Antibiotic	Range of dilution (mg/L)	*C. jejuni* (mg/L) R >	Resistance in human isolates (%) (n= 59)	Resistance in poultry isolates (%) (n = 48)
Erythromycin	0.25–512	4	5.1	2.1
Ciprofloxacin	0.03–64	0.5	72.9	68.8
Tetracycline	0.125–256	1	52.5	27.1
Streptomycin	0.25–512	4	23.7	12.5
Gentamicin	0.125–256	2	0	0
Chloramphenicol	0.125–256	16	0	0
Ampicillin*	0.06–128	4	67.8	54.2
Nalidixic acid	1–128	16	62.7	58.3

R = resistant
n = number of tested isolates
*The parameters were adopted from Communique (2005), the parameters for other antibiotics were based on recommendations issued by the EU Reference Laboratory (EURL-AR, 2012).

their identification was confirmed by MALDI-TOF MS. Quality control was performed using the *C. jejuni* reference strain ATCC 33560.

Detection of virulence genes

To test the presence of the particular virulence genes, a total of 94 human isolates (*C. jejuni* – 73, *C. coli* – 21) and 123 food isolates (*C. jejuni* – 70, *C. coli* – 53) were selected. The food isolates were only from poultry and from pork liver as no *Campylobacter* spp. were detected in milk samples. Genetic detection of 9 selected virulence genes which play a part in *Campylobacter* virulence was performed using PCR with a set of specific primers for determining the presence of the *cdtA*, *cdtB*, *cdtC*, *virB11*, *wlaN*, *ciaB*, *iam*, *dnaJ* and *racR* genes. The PCR conditions for all the above genes have been previously described [5].

Antibiotic susceptibility

In confirmed *Campylobacter* spp. isolates, resistance to selected antimicrobial agents was tested with the microdilution method [16]. The tests were performed on microtitration plates in solutions of the particular antibiotics and Mueller-Hinton broth with 2.5% lysed horse blood (Trios). The inoculated plates were incubated in a microaerophilic atmosphere (GENbox microaer, BioMérieux) at 37 °C for 48 hours. Resistance to the following selected antibiotics was tested: erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin, chloramphenicol, ampicillin and nalidixic acid. The parameters for individual antibiotics, including interpretation criteria, were based on recommendations issued by the EU Reference Laboratory – Antimicrobial Resistance and Antibiogram Committee of the French Microbiology Society [17, 18]. Quality control was performed at regular intervals using the *C. jejuni* reference strain ATCC 33560. The above approach was used to test 82 human isolates (*C. jejuni* – 59, *C. coli* – 23) and 91 food isolates (*C. jejuni* – 48, *C. coli* – 43). The detailed parameters for testing are shown in Tables 1 and 2.

Detection of antibiotic resistance genes

The isolates with antibiograms were tested for the presence of *blaOXA-61*, *tet(O)*, *aph*-3-1 and cmeB genes. These genes were detected using PCR with specific primers [19]. These tests were carried out in 59 human, 48 poultry and 2 pork liver isolates of *C. jejuni* and 23 human, 30 poultry and 13 pork liver isolates of *C. coli*. Given the small number of *C. jejuni* isolates obtained from pork liver, the relationship between phenotypic and genotypic resistance was not further assessed in this subgroup.

Statistical analysis

Fisher's exact test was used to compare the frequencies of virulence genes in *C. jejuni* and *C. coli* in human and poultry isolates. The same test was used to compare the frequencies of the bla_{OXA-61} gene in ampicillin-susceptible and -resistant human and poultry isolates as well as of the *tet(O)* gene in tetracycline-susceptible and -resistant isolates from patients, poultry and pork liver. For multiple comparisons, Fisher's exact test with Bonferroni correction was used. IBM SPSS Statistics version 22 was used to analyze the data. A significance level less than 0.05 was considered statistically significant ($p < 0.05$).

Table 2. The parameters of testing and resistance to the selected antibiotics in *C. coli*

Antibiotic	Range of dilution (mg/L)	C. coli (mg/L) R >	Resistance in human isolates (%) (n= 23)	Resistance in poultry isolates (%) (n=30)	Resistance in pork liver isolates (%) (n=13)
Erythromycin	0.25 – 512	8	8.7	3.3	23.1
Ciprofloxacin	0.03 – 64	0.5	69.6	66.7	61.5
Tetracycline	0.125 – 256	2	34.8	56.7	84.6
Streptomycin	0.25 – 512	4	26.1	56.7	92.3
Gentamicin	0.125 – 256	2	0	0	7.7
Chloramphenicol	0.125 – 256	16	0	3.3	0
Ampicillin*	0.06 – 128	4	47.8	56.7	53.8
Nalidixic acid	1 – 128	16	52.2	56.7	61.5

R = resistant
n = number of tested isolates
*The parameters were adopted from Communique (2005), the parameters for other antibiotics were based on recommendations issued by the EU Reference Laboratory (EURL-AR, 2012).

Table 3. Virulence genes in *Campylobacter* spp. isolated from humans and foods

Genes	Percentage of positive human isolates		Percentage of positive food isolates			
	C. jejuni (n = 73)	*C. coli* (n = 21)	*C. jejuni* from poultry (n = 67)	*C. coli* from poultry (n = 36)	*C. jejuni* from pork liver (n = 3)	*C. coli* from pork liver (n = 17)
cdtA	94.5	0	90.1	0	100	5.9
cdtB	95.9	9.5	100	2.8	100	5.9
cdtC	98.6	9.5	100	2.8	100	5.9
virB11	0	0	4.4	0	0	5.9
ciaB	61.6	0	60	0	100	0
wlaN	4.1	0	2.3	0	0	0
iam	0	95.2	0	97.2	0	82.4
dnaJ	94.5	0	96.6	0	100	5.9
racR	84.9	9.5	95.6	2.8	100	5.9

n = number of tested isolates

RESULTS

Prevalence of virulence genes

A total of 56% of poultry samples (68% of fresh chicken, 44% of frozen chicken) were found to contain *Campylobacter* spp. Pork liver samples were contaminated in 24% of cases. The raw cow's milk samples were negative. *Campylobacter*s identified in individual foods showed species specificity. While *C. jejuni* was more prevalent in poultry meat (67.2% of isolates), *C. coli* prevailed in pork liver (80.0% of isolates). Among 235 human isolates, most belonged to *C. jejuni* (88.9%). That is why the tested human isolates of *C. coli* are low in numbers. The percentages of the virulence genes in selected human and food *C. jejuni* and *C. coli* isolates are shown in Table 3.

The results suggest that the studied virulence genes are more prevalent in *C. jejuni* than in *C. coli*. This is particularly apparent in genes encoding CDT, which were detected in the vast majority of the tested isolates of *C. jejuni* obtained from both humans and foods. Most *C. jejuni* isolates carried 5 or 6 virulence genes simultaneously (83.6% of human isolates, 90.0% of food isolates), as opposed to *C. coli* isolates in which, with a few exceptions, only the *iam* gene was detected (85.7% of human isolates, 88.7% of food isolates). There were no considerable differences in the frequency of most virulence genes between isolates of the same species of the tested *Campylobacter*s obtained from humans and foods. The only statistically significant difference in the prevalence of virulence genes was noted in case of *racR* in *C. jejuni*. The prevalence was higher in food isolates (84.9% of human isolates vs. 95.6% of food isolates; p = 0.046).

Antimicrobial resistance

Phenotypic resistance to the selected antibiotics is shown in Tables 2 and 3, clearly showing high resistance to quinolone antibiotics and ampicillin in both tested *Campylobacter* species. Moreover, the tested *C. coli* isolates showed high resistance to streptomycin and increased resistance to erythromycin (as much as 23% in case of pork liver isolates). Only 14 (8%) out of the 175 tested *Campylobacter* spp. samples were susceptible to all antibiotics. The data of multiple antimicrobial resistance are shown in Table 4.

Prevalence of resistance genes and the genotype-phenotype relationship

The testing of phenotypic resistance to antibiotics was followed by detecting the selected resistance genes (bla_{OXA-61}, *tet(O)*, *cmeB* and *aph3-1*). The most frequent gene was bla_{OXA-61}, detected in 74.6% and 74.0% of the tested *C. jejuni* isolates obtained from humans and foods, respectively. In case of *C. coli* isolates, the same gene was detected in 78.3% of human isolates and 67.4% of food isolates. The gene was more prevalent in isolates resistant to ampicillin (*C. jejuni* 87.5% of human isolates and 84.6% of food isolates; *C. coli* 90.9% and 97.7%, respectively). Comparison of the frequencies of the *blaOXA-61* gene between ampicillin-susceptible and -resistant human isolates of *C. jejuni* revealed that the gene was significantly more common in ampicillin-resistant human isolates (p = 0.003). The difference was not significant in poultry isolates (p = 0.302). The opposite was true for ampicillin-resistant isolates of *C. coli*. The difference was not significant in human isolates (p = 0.317). The bla_{OXA-61} gene

Table 4. Antimicrobial resistance of Campylobacter isolates to multiple antibiotics

Number of antibiotics	Number of isolates (%)			
	human isolates		food isolates	
	C. jejuni (n = 59)	*C. coli* (n = 23)	*C. jejuni* (n = 50)	*C. coli* (n = 43)
0	3 (5.1)	3 (13.6)	7 (14.0)	1 (2.3)
1	11 (18.6)	2 (9.1)	11 (22.0)	3 (7.0)
2	6 (10.2)	10 (40.9)	13 (26.0)	10 (23.3)
3	15 (25.4)	3 (13.6)	10 (20.0)	9 (20.9)
4	23 (39.0)	3 (13.6)	6 (12.0)	13 (30.2)
5	0 (0.0)	2 (9.1)	3 (6.0)	6 (14.0)
6	1 (1.7)	0 (0.0)	0 (0.0)	1 (2.3)

n = number of tested isolates

was significantly more frequent in ampicillin-resistant isolates of *C. coli* obtained from pork liver and poultry ($p = 0.029$ and $p = 0.009$, respectively). The *tet(O)* gene, responsible for resistance to tetracycline, was detected in 37.3% of human and 20.0% of food isolates of *C. jejuni* and 34.8% of human and 39.5% food isolates of *C. coli*. The rates were higher in isolates with phenotypic resistance (*C. jejuni* 58.1% and 76.9%, respectively; *C. coli* 87.5% and 57.1%, respectively). The *tet(O)* gene was significantly more frequent in tetracycline-resistant isolates of *C. jejuni* obtained from both patients and poultry ($p = 0.001$ and $p < 0.0001$, respectively). Similarly, there was a significant difference in tetracycline-resistant human and poultry isolates of *C. coli* ($p < 0.0001$ and $p = 0.042$, respectively). The difference was not significant in pork liver isolates of *C. coli* ($p = 0.128$). The cmeB gene (efflux pump) was detected in only one human isolate of *C. jejuni* but in 91.3% of human and 76.7% of food isolates of *C. coli*. The *aph*3-1 gene was detected in one human *C. jejuni* isolate and in one *C. coli* isolate obtained from pork liver. Relationships between the presence of selected genes responsible for mechanisms of resistance linked to particular antibiotics and phenotypic resistance to the antibiotics are shown in Tables 5 and 6. It is apparent from the tables that the assessed resistance genes are more frequent in isolates with phenotypically determined resistance. This is particularly true for *Campylobacter* spp. resistant to tetracycline which carried the *tet(O)* gene significantly more frequently that isolates susceptible to the antibiotic.

DISCUSSION

This study provides the first insights into the prevalence of nine virulence genes important in the pathogenesis of *C. jejuni* and *C. coli* in the Czech Republic. In the tested *C. jejuni* isolates, the most prevalent virulence genes were those responsible for the production of CDT (90–100%). But their prevalence in *C. coli* isolates was low (up to 10%). High prevalence rates of these genes in *C. jejuni* isolated from both humans and poultry (100%) were also reported by Datta et al. [5] or Ripabelli et al. [20]. Slightly lower rates of prevalence of *cdtA* (64%), *cdtB* (82%) and *cdtC* (84%) in poultry isolates of *C. jejuni* were found by Polish authors who, in contrast to the present study, also reported high rates of these genes in *C. coli* isolates (*cdtA* – 100%, *cdtB* – 91% and *cdtC* – 100%) [21]. However, another Polish study found CDT genes in only 5.6% of human isolates of *C. coli* [22]. Genes responsible for the invasiveness of *Campylobacter* include *virB11*. In the present study, the gene was detected in 4% of poultry isolates of *C. jejuni* and 6% of *C. coli* isolates obtained from pork liver. Krutkiewicz and Klimuszko [21] identified the gene in 14% of *C. jejuni* and 9% of *C. coli* isolates from poultry and 42% and 0% of pig isolates, respectively. Another gene, *ciaB*, was only detected in *C. jejuni* isolates (humans – 62%, poultry – 60% and pork liver – 100%) but in none of *C. coli* isolates from either patients or foods. Datta et al. [5] found the gene in 98% of human and 100% of poultry isolates of *C. jejuni*. The gene *wlaN* contributing to post-infection complications in the form of Guillain-Barré syndrome was only detected in *C. jejuni*, namely in 4% of human isolates and 2% of poultry isolates. Cha et al. [23] found the gene in 35% of *C. jejuni* isolates obtained from Korean patients with *Campylobacter*iosis and 23% of isolates from Southeast Asia. Feodoroff et al. [24] identified the gene in 23% of 166 human isolates of *C. jejuni*. Talukder et al. [6] found the *wlaN* gene in 9% of samples in a set of 40 human isolates of *C. jejuni*. Datta et al. [5] performed a study of virulence factors in clinical human isolates, poultry meat, broiler feces and bovine feces. The detection rates for the *wlaN* gene were 25.0%, 23.8%, 4.7% and 7.7%, respectively. In the present study, the *iam* gene was only confirmed in *C. coli* isolates (humans – 92%, poultry – 97% and pork liver – 82%). Wieczorek and Osek [25] stated that the gene was present in 31% of *C. jejuni* isolates and 27% of poultry isolates of *C. coli*. In another study, Wieczorek [26] reported 100% prevalence of the gene in poultry isolates of *C. jejuni* and 15% prevalence in *C. coli* isolates. The considerable difference in the genetic makeup of *C. jejuni* and *C. coli* strains tested in the present study was also apparent in case of the genes *dnaJ* and *racR* detected in the majority of *C. jejuni* isolates (85–100%). In *C. coli*, the *dnaJ* gene was found in 6% of isolates from pork liver; the *racR* gene was detected in 9% of *C. coli* isolates from patients, 3% of poultry isolates and 6% of isolates from pork liver. In a study by Thakur et al. [27], for instance, the *dnaJ* gene was present in approximately 16% of human and 11% of poultry isolates of *C. jejuni*. The authors found *dnaJ* in 72% of poultry *C. coli* isolates but failed to detect the gene in human isolates of *C. coli*. It is therefore clear that the prevalence of genes responsible for virulence and production of toxins is rather varied in thermotolerant *Campylobacter* spp. This fact was also shown in a study by Wieczorek et al. [28] comparing the presence of 8 selected virulence genes in *Campylobacter* isolates from Poland, Australia and Malaysia. The genes differed in prevalence, depending on the country of

Table 5. Comparison of genotypic and phenotypic resistance to ampicillin and tetracycline in *C. jejuni* isolates

Origin of isolates	Presence of the bla_{OXA-61} gene in *C. jejuni* isolates (%)		Presence of the *tet(O)* gene in *C. jejuni* isolates (%)	
	AMP-R isolates	AMP-S isolates	TET-R isolates	TET-S isolates
humans	87.5	47.7	58.1	14.3
poultry	84.6	68.2	76.9	0

Table 6. Comparison of genotypic and phenotypic resistance to ampicillin and tetracycline in *C. coli* isolates

Origin of isolates	Presence of the bla_{OXA-61} gene in *C. coli* isolates (%)		Presence of the *tet(O)* gene in *C. coli* isolates (%)	
	AMP-R isolates	AMP-S isolates	TET-R isolates	TET-S isolates
humans	90.9	66.7	87.5	6.7
poultry	94.1	46.1	47.0	7.7
pork liver	85.7	16.7	72.7	0

AMP-R: isolate with phenotypic resistance to ampicillin
AMP-S: isolate with phenotypic susceptibility to ampicillin
TET-R: isolate with phenotypic resistance to tetracycline
TET-S: isolate with phenotypic susceptibility to tetracycline

origin. Genetic diversity is definitely influenced by geographical location and sources of isolates, including climate, approach to agriculture and use of antibiotics. For example, five of the genes (*ciaB*, *racR*, ceuE, *cdtB* and cdt gene cluster) were least prevalent in isolates from Malaysia. However, the authors also pointed to genetic diversity in the subgroup of isolates from Poland which could not be clearly explained.

Another parameter analyzed in the present study was phenotypic resistance of thermotolerant *Campylobacter* spp. isolated from foods and humans to eight selected antibiotics. Europe is typically characterized by high resistance of *Campylobacter* spp. to quinolones. For poultry isolates of *C. jejuni*, the 2012 rates of resistance to ciprofloxacin were 89% in Poland, 82% in Hungary, 81% in Romania and 63% in Austria. The mean resistance to ciprofloxacin in the EU was 60% in the same year. The mean rate of resistance to nalidixic acid calculated from data provided by individual countries was 58% [9]. In the present study, the resistance of poultry isolates of *C. jejuni* to ciprofloxacin and nalidixic acid was 69% and 58%, respectively; for *C. coli* isolates, the rates were 67% and 58%, respectively. For isolates of *C. coli* obtained from pork liver, the resistance to both quinolones was roughly identical at approximately 61%. Comparison of resistance of *C. coli* isolates obtained from poultry and pork liver to tetracycline (57% vs. 85%) and streptomycin (57% vs. 92%) showed higher rates in pig isolates. This may be explained by the fact that tetracycline antibiotics are more frequently used in pig farming. In 2012, high resistance of pig *C. coli* isolates to tetracycline was reported, for example, in Spain (100%), France (92%) or Hungary (89%) [9]. Since macrolides are the drug of choice when antibiotic therapy of human *Campylobacter*iosis is needed, attention was paid to testing resistance to erythromycin. In the present study, 2% of poultry isolates of *C. jejuni*, 3% of poultry isolates of *C. coli* and 23% of *C. coli* isolates from pork liver were resistant to erythromycin. Human isolates of *C. jejuni* were more resistant to all antibiotics than poultry isolates. By contrast, human isolates of *C. coli* were less resistant to most antibiotics than food isolates; the only exception was ciprofloxacin, with there being a slightly higher resistance of human isolates to this antibiotic (70% vs. 61% and 68%, respectively). Resistance of human isolates of *C. coli* to erythromycin (9%) was higher than that of poultry isolates (3%) but lower than resistance of isolates obtained from pork liver (23%).

Antibiotic resistance is encoded by numerous genes that may, but do not have to, be expressed in the form of phenotypic resistance tested *In vitro* by, for example, the microdilution method and manifested in vivo by failed antibiotic therapy. The present study focused on detecting of four genes, three of which (*blaOXA-61*, *tet(O)* and *aph*3-1) are thought to be associated with resistance to a particular group of antibiotics and one is linked to efflux pump activity (cmeB). The *aph*3-1 gene was only detected in two isolates. In another two genes encoding the mechanism of resistance linked to a particular antibiotic, the study tried to assess relationships between the presence of a particular gene and phenotypic resistance to that antibiotic. Those were the *blaOXA-61* gene linked to resistance to ampicillin and the *tet(O)* gene linked to resistance to tetracycline (see Tables 5 and 6). In all cases, the genes were more frequent in resistant isolates as compared with susceptible isolates. This was particularly true for the *tet(O)* gene participating in protection of bacterial ribosomes against the effects of tetracyclines. For instance, the gene was highly prevalent in resistant isolates of *C. jejuni* from poultry and *C. coli* from pork liver (77% and 73%, respectively) but it was not detected in similar isolates susceptible to tetracycline. Abdi-Hachesoo et al. [29] detected the *tet(O)* gene in as many as 93% of *C. coli* isolates and 74% of *C. jejuni* isolates from poultry. In the present study, less striking differences in the prevalence of the *tet(O)* gene were found between resistant and susceptible human isolates of *C. jejuni*. Isolates resistant and susceptible to tetracycline carried the *tet(O)* gene in 58% and 14%, respectively.

In accordance with detection of high phenotypic resistance to ampicillin, high prevalence rates of the *blaOXA-61* gene contributing to resistance to this antibiotic were found in both *Campylobacter* species. The prevalence rates of the gene in both *C. jejuni* and *C. coli* isolated from both foods and humans were relatively similar, ranging from 67% to 78%. Although the *blaOXA-61* gene was more frequently detected in ampicillin-resistant isolates (88% vs. 56%), its presence is not necessarily linked with resistance [30, 31]. This is confirmed by the fact that in the present study, the gene was detected in 48% of susceptible *C. jejuni* isolates and 67% of susceptible *C. coli* isolates obtained from humans. The *blaOXA-61* gene was also carried by 68% of poultry isolates of *C. jejuni*.

The remaining gene, cmeB, is responsible for mechanisms potentially causing resistance of bacteria to a broader range of antibiotics. Therefore, it is more difficult to determine its significance with respect to phenotypic resistance to a particular antibiotic group. This gene encodes the inner membrane transporter which is a part of efflux pump and can be associated with resistance to several antibiotic classes such as quinolones [12]. Although the microdilution method showed high resistance of both *Campylobacter* species to quinolones, the prevalence of the cmeB gene in *C. jejuni* isolates was rather low (2% in human isolates; undetected in food isolates). This observation is in accordance with previously published data suggesting that resistance to quinolones is primarily associated with other resistance mechanisms, especially mutation in genes encoding DNA gyrase and DNA topoisomerase [10, 32]. Higher prevalence of cmeB in *C. coli* than *C. jejuni* isolates was also observed by Obeng et al. [19]. But this fact may be related to higher sequence variability of the cmeB gene in the tested isolates.

CONCLUSION

In conclusion, high percentages of both human and food isolates of *C. jejuni* carried the toxigenic genes *cdtA*, *cdtB* and *cdt*C. Conversely, the prevalence of these genes in *C. coli* isolated from all types of samples included in the study was low. The genes *ciaB*, *dnaJ* and *racR* were found to be highly prevalent in both human and food isolates of *C. jejuni*. The studied virulence genes were considerably more prevalent in *C. jejuni* isolates than in *C. coli* isolates. This fact was particularly apparent in the

dnaJ and *racR* genes. The only exception was the *iam* gene identified in only *C. coli*.
The tested isolates of both *C. jejuni* and *C. coli*, irrespective of their origin, showed high levels of phenotypic resistance to mainly quinolone antibiotics as well as higher levels of resistance to ampicillin, tetracycline and streptomycin. *C. coli* isolates from pork liver had 23% resistance to erythromycin. Both *Campylobacter* species showed high prevalence rates of *blaOXA-61* and *tet(O)*, genes encoding resistance to antibiotics. There were considerable differences in the prevalence of the cmeB gene between *C. jejuni* and *C. coli*. There was a significant difference between a high prevalence of the resistance-encoding gene *tet(O)* in tetracycline-resistant isolates and its low prevalence in isolates susceptible to the drug. The present study is the first in the Czech Republic to assess the prevalence of virulence and antibiotic resistance genes in *Campylobacter* isolated from humans and foods.

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