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Classical and Molecular Identification of Thermotolerant Campylobacters from Poultry Meat

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Summary

Poultry meat samples from Slovenian retail market were examined for the presence of thermotolerant campylobacters. The isolates were identified by phenotypic and genotypic methods. ISO 10272 recommendations were followed for phenotypic identification. Different PCR assays, targeting species specific DNA regions in *C. jejuni* and *C. coli*, were checked for their applicability in identification. High degree of tested samples was positive (27/33), with significant proportion of *C. coli* (32 %) among identified strains. High percentage of *C. jejuni* strains (54 %) were hippurate negative. Phenotypic identification was therefore found to be inconvenient because of the presence of the strains with atypical phenotype and possible misinterpretation of test results. Multiplex PCR, targeting hippuricase gene in *C. jejuni* and species specific region in *C. coli*, was found to be an efficient method that allowed fast, simple and accurate identification of *C. jejuni* and *C. coli*. *FlaA* PCR is a reliable method to identify the group *C. jejuni*/*C. coli*, but it does not differentiate between the two species. *CdtB* PCR is inconvenient because of many false negative and some false positive results.

Key words: poultry meat, *Campylobacter*, *C. jejuni*, *C. coli*, phenotypic identification, genotypic identification, PCR, *flaA* gene, *cdtB* gene, hippuricase gene

Introduction

Campylobacter spp. are one of the most common bacterial causes of acute diarrhoea in humans throughout the world. The incidence of *Campylobacter* infections continues to rise and it already exceeds the number of salmonellosis. The thermotolerant *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* are the most important species, with *C. jejuni* and *C. coli* accounting for the majority of human infections (*1*). Numerous vehicles for transmission of *Campylobacter* infections have been identified. Raw milk, untreated surface water, and poultry meat have been shown to be major sources of *C. jejuni* and *C. coli* infections (*2*,*3*).

Many methods have been proposed for detecting thermotolerant *Campylobacter* in faeces, water and food products (*4*,*5*). A horizontal method ISO 10272 (*6*) was standardised in 1996 in order to correlate laboratory results obtained for food used in international trade (*5*). Traditional phenotypic tests for differentiation and species identification of campylobacters take 5–7 days and are often problematic because campylobacters have fastidious growth requirements and few distinguishing biochemical characteristics. Identification is also hindered by subjective interpretation of biochemical test results and because some isolates have atypical phenotypes. For example, the differentiation between *C. jejuni*

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and *C. coli* relies on the ability of *C. jejuni* to hydrolyse hippurate, but certain atypical *C. jejuni* strains fail to do so (*7*). These limitations might be overcome by the use of PCR-based identification methods. Currently, a number of PCR protocols, targeting different genes within the genus *Campylobacter*, or specifically within the species *C. jejuni* and *C. coli*, have been developed and applied for detecting and identifying these organisms (*8,9*). Linton *et al.* (*10*) developed a PCR assay specific for the genus *Campylobacter* as well as PCR assays specific for *C. jejuni* and *C. coli* (*8*). The flagellin genes can serve as a good epidemiological marker and several *flaA* typing procedures have been developed with considerable variations in DNA preparation technique, primer design and annealing temperatures. To allow the direct comparison of results obtained in different laboratories the consensus pair of primers has been developed for identification and typing of *C. jejuni* and *C. coli* strains (*11*). The amplification of genes for cytolethal distending toxin (CDT) was also used to identify *C. jejuni* and *C. coli* with further restriction analysis allowing the differentiation between the two species (*12*).

In Slovenia, the increasing incidence of campylobacteriosis was reported from the regular surveillance system in the last years. In the year 2000, 67.3 cases of campylobacteriosis per 100 000 habitants were reported. High percentage of patients (35 %) were hospitalised (*13*). Handling and consumption of the contaminated undercooked poultry meat is suspected to be an important source of *Campylobacter* infections. However, no data on the extent of poultry meat contamination with *Campylobacter* spp. in Slovenia are available.

The aims of our study were (*i*) to compare classical phenotypic and genotypic (PCR) identification of thermotolerant campylobacters from poultry meat; (*ii*) to evaluate the applicability of different PCR systems for identifying *C. jejuni* and *C. coli* from poultry meat samples.

Materials and Methods

Sampling

33 samples of poultry meat products (chicken liver and legs) coming from 5 different suppliers on the Slovenian market were investigated for the presence of thermotolerant campylobacters. Five samplings were done between September 2000 and May 2001.

In 4 samplings, samples from 3 suppliers were investigated. From each supplier one sample of chicken liver and one sample of chicken legs were taken. In one sampling, the samples were taken from 5 suppliers. From each of them we took one sample of chicken legs, while chicken liver was tested only from 4 of those suppliers.

Isolation procedures for Campylobacter spp.

The standardised procedure, recommended by ISO 10272 standard, was followed. Thermotolerant campylobacters were isolated from 5 g of food (chicken liver or skin from legs). The bacteria were enriched in 45 mL of selective Preston broth (Oxoid), containing 5 % of horse blood (SR 048C, Oxoid) and incubated for 18 hours at 42 °C in OCN atmosphere (5 % O_2, 10 % CO_2, 85 % N_2). One loopful of enrichment broth was streaked on charcoal cefoperazone deoxycholate (CCDA, Oxoid) and Karmali (Biokar diagnostics) selective agars and incubated microaerobically in gastight containers at 42 °C for 3 days (ISO 10272).

Species determination

Five colonies from each culture plate, suspected by their colony morphology to be *Campylobacter* spp., were Gram stained. If small curved rods were observed, the colony was enriched in Brucella broth, streaked on Columbia selective agar plates (selective supplement SR 069C, Oxoid) and identified by conventional tests as described in ISO 10272. The isolated culture was checked for aerobic growth, growth at 25 °C, motility, presence of oxidase and catalase, fermentation of sugars and the hydrolysis of hippurate. For long term preservation isolates were resuspended in brain heart infusion broth and stored at –70 °C in 20 % glycerol.

For further analysis DNA was isolated from the colonies using two methods; (*i*) a classical procedure where DNA is extracted with the use of guanidinium thyocyanate (*14*), and (*ii*) a simple boiling procedure (*15*).

PCR and RFLP analysis

Four different PCR assays were applied. Two of them, amplification of flagellin (*flaA*) and cytolethal distending toxin (*cdtB*) gene loci, identify the group *C. jejuni/C. coli*, whereas the multiplex PCR discriminates between the two species. The fourth PCR was used for *Campylobacter* identification on the genus level. PCR reagents were obtained from Promega. All reactions were performed using a GeneAmp PCR System 2400 (Perkin Elmer) in a total volume of 25 μL, and 1 μL aliquot of the bacterial or DNA extract was used as a template. In all PCR experiments, negative controls for PCR reactions and sample preparations were included. The amplified product (2 μL) was analysed by agarose gel electrophoresis using 1 % (w/v) agarose in 0.5 × TAE. A 100 bp DNA ladder was used as a standard for molecular size determinations (Gibco). The fragments were stained with ethidium bromide and photographed using an UV transilluminator to visualise the bands.

FlaA PCR

The consensus pair of primers, recommended for *flaA* amplification was used (*11*), while the other PCR reagents and conditions of amplification were chosen according to Nachamkin (*15,16*).

Multiplex PCR

The identification of *C. jejuni* and *C. coli* was done by JEJ1 (5'- GAA GAG GGT TTG GGT GGT G -3') – JEJ2 (5'- AGC TAG CTT CGC ATA ATA ACT TG -3') for *C. jejuni* with the annealing temperature of 63 °C and by COL1 (5'- GGT ATG ATT TCT ACA AAG CGA G -3') – COL2 (5'- ATA AAA GAC TAT CGT CGC GTG -3') for *C. coli* with the same annealing temperature of 63 °C . The primer pair JEJ1-JEJ2 is directed to hippuricase gene in *C. jejuni* and gives an amplicon of 735 bp. The primers COL1-COL2 were designed based on the sequence

of the clone that hybridised to *C. coli* strains. They generate a *C. coli* specific amplicon of 500 bp (*8*). Both pairs of primers were used in the same reaction (*17*).

CdtB PCR

The amplification conditions and concentrations of all the reagents in the PCR reaction were used as previously described (*12*).

»Genus specific PCR«

In this PCR assay the genus *Campylobacter* specific region of 16S rRNA gene sequence is amplified by C412F and C1288R primers (*10*).

RFLP analysis of *cdtB* gene

This analysis was used to discriminate between the species *C. jejuni* and *C. coli*. *Eco*RI cuts the relevant portion of *C. jejuni cdtB* gene, but not the *C. coli cdtB* gene (*12*). A portion of the PCR product of *cdtB* gene was digested with the restriction endonuclease *Eco*RI (Promega R 6011) according to the manufacturer's instructions. The digestion was stopped with 2 μL of DNA loading buffer. The digested DNA (10 μL) was analysed by gel electrophoresis using 3 % (w/v) agarose in 0.5 × TAE.

Results

Isolation of strains from contaminated samples

Thirty-three samples of poultry meat (chicken legs and liver) from 5 different Slovenian suppliers on the retail market were examined for the presence of thermotolerant campylobacters in a period of nine months between September 2000 and May 2001. High degree of tested samples (27/33) was positive for campylobacters, with similar proportions of contaminated chicken legs (n=13) and chicken liver (n=14). Higher degree of contaminated samples was found at samplings in early autumn (15/15), than in early spring (12/18).

Phenotypic and genotypic identification of isolates

In total, 163 presumptive thermotolerant C*ampylobacter* strains were isolated from CCDA and Karmali agar plates following the ISO 10272 recommendations. They were all Gram negative, narrow, spiral-shaped, nonspore-forming bacilli in microscopic preparations. For species identification the isolates were enriched in Brucella broth and streaked onto Columbia agar plates, where the pure culture was grown after 6 days from the beginning of the procedure. Phenotypic and genotypic methods were used for species identification.

Phenotypic identification

By standard phenotypic tests, 95 isolates were initially identified as members of thermotolerant *Campylobacter* species. ISO recommendations were used again for classical identification. Growth in microaerobic atmosphere (5 % oxygen) in gastight containers at 42 °C, the absence of growth aerobically and at 25 °C, oxidase and catalase activity but the absence of glucose, sucrose and lactose fermentation were required specifities of thermotolerant campylobacters. A hydrolysis of hippurate was a biochemically distinguishing feature of *C. jejuni* against *C. coli* strains. Among 163 presumptive isolates, 68 were identified by phenotyping as species other than thermotolerant campylobacters.

Genotypic identification

Initially, two genotypic assays multiplex PCR and *flaA* PCR were applied for genotypic identification. At least with one of the two applied PCR assays, the regions specific for *C. jejuni* or/and *C. coli*, were found at 106 isolates. At *flaA* PCR, an amplified fragment (1700 bp) was specific for *C. jejuni* and *C. coli* (Fig. 1). In the case of multiplex PCR, we identified *C. jejuni* if the amplified fragment with 735 bp was obtained and *C. coli* if the amplicon was of 500 bp (Fig. 2).

Among 163 presumptive isolates, for 57 isolates no amplification product was obtained with any of the two PCR assays used.

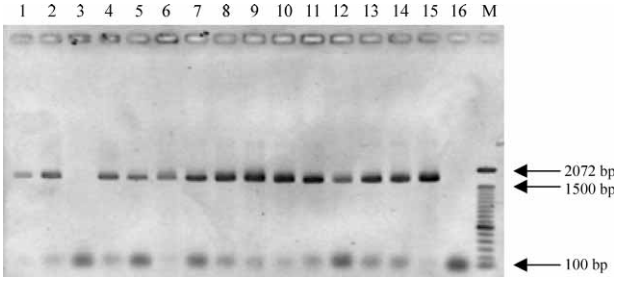

Fig. 1. PCR amplification of *flaA* gene fragment, present in *C. jejuni* and *C. coli*. In lanes 1, 2 and 4–15 the specific PCR product of 1700 bp was obtained. M: 100 bp DNA ladder, Gibco.

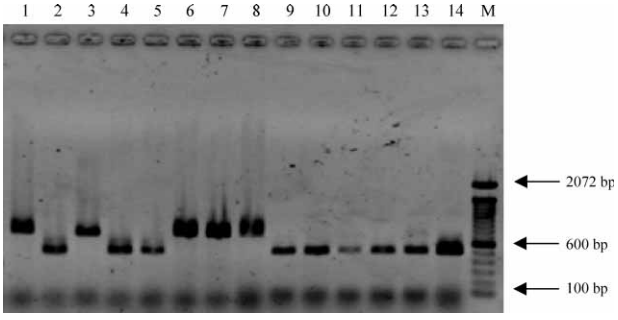

Fig. 2. Amplification of specific regions in *C. jejuni* and *C. coli* by multiplex PCR. In lanes 1, 3 and 6–8 the specific PCR product (735 bp) for *C. jejuni* was obtained, and in lanes 2, 4, 5 and 9–14 the specific PCR product (500 bp) for *C. coli*. M: 100 bp DNA ladder, Gibco.

The comparison between phenotypic and genotypic identification

Standard phenotypic tests and genotypic assays (*flaA* PCR, multiplex PCR) gave the same result for 113 (69.3 %) isolates.

When standard phenotypic tests and at least one of the two genotypic assays gave positive result, the isolate was considered as *C. jejuni/C. coli* and that was the case for 79 (48.5 %) isolates. For 34 (20.8 %) isolates both standard phenotyping and the two PCR assays showed

Table 1a. The comparison between phenotypic and genotypic identification of presumptive thermotolerant campylobacters isolated from selective CCDA and Karmali agars

Total number of isolates	**163**	**Identification**
– SP positive – PCR (*flaA* or multiplex) positive	79	*C. jejuni*/*C. coli*
– SP negative – PCR (*flaA* and multiplex) negative – Genus specific PCR negative	34	non-campylobacters
– Different result with SP and PCR (*flaA* and multiplex) – Genus specific PCR positive	50	campylobacters – further analysis in Table 1b

SP standard phenotyping
PCR Polymerase Chain Reaction

Table 1b. The identification of *Campylobacter* strains (all were positive by genus specific PCR) where different results were obtained with standard phenotyping (SP) and PCR (*flaA* and multiplex)

Total number of isolates	**50**	**Identification**
– SP negative (misinterpretation of phenotypic tests) – PCR (*flaA* or multiplex) positive	27	*C. jejuni*/*C. coli*
– SP positive – PCR (*flaA* and multiplex) negative	16	thermotolerant campylobacters, but not *C. jejuni*/*C. coli*
– SP negative – PCR (*flaA* and multiplex) negative – genus specific PCR positive	7	non thermotolerant campylobacters

SP standard phenotyping
PCR Polymerase Chain Reaction

negative results and those strains were considered as non-*C. jejuni*/*C. coli*. These isolates were later tested as negative by the genus specific PCR (*10*) and thereafter considered as non-campylobacters. The remaining 50 (30.7 %) isolates were positive either by phenotypic or genotypic method, but not with both (Table 1a). They were tested by genus specific PCR , while the results of phenotypic tests were re-evaluated for the possible misinterpretation of motility and oxidase test results. All those isolates were identified as campylobacters because a genus-specific amplicon of 816 bp was obtained. Twenty-seven (16.6 %) isolates were found to be *C. jejuni*/*C. coli*, but they were initially misidentified with phenotypic tests because of mistakes made by interpretation of the tests (n=10) or because the culture was not pure (n=17). Sixteen (9.8 %) isolates were found to be the members of other thermotolerant *Campylobacter* species, while for 7 (4.3 %) isolates we could conclude they were the members of the genus *Campylobacter*, but present in a mixed culture (Table 1b).

The evaluation of applicability of different PCR systems for identifying C. jejuni and/or C. coli

Initially, two genotypic assays multiplex PCR and *flaA* PCR were applied and amplicons of 106 isolates were achieved.

Eighty-two isolates were amplified by both assays and 19 isolates only by multiplex PCR and those isolates were identified as *C. jejuni* or *C. coli*. For 5 isolates, amplicons only with *flaA* PCR were achieved and they were referred as *C. jejuni*/*C. coli*. For 57 isolates no amplification product was obtained with any of the two PCR assays used (Table 2).

CdtB amplification and species identification

Additionally, we wanted to test the applicability of PCR assay to amplify the *cdtB* genes present in *C. jejuni* and *C. coli* and the power of restriction analysis for discrimination between the two species (Fig. 3). All the presumptive thermotolerant *Campylobacter* colonies were tested and amplicons were obtained from 69 isolates. Those isolates were confirmed as *C. jejuni*/*C. coli* by multiplex PCR or *flaA* PCR as well as by phenotypic tests. Five isolates that were positive by *cdtB* PCR were negative by other applied genotypic and phenotypic methods, so we considered the results of *cdtB* amplification as false positive (Table 2).

Species identification of C. jejuni and C. coli

Three methods were used to differentiate between the species *C. jejuni* and *C. coli*, the conventional test of the hydrolysis of the hippurate, multiplex PCR and restriction analysis of the *cdtB* amplicon. Of 106 identified isolates by PCR (Table 2), 101 isolates could be identified according to the results of the three methods. For the remaining 5 isolates species identification could not be made, because the result of the hippurate reaction was

Table 2. The efficiency of different PCR systems for identification of *C. jejuni* and *C. coli*

	***flaA* PCR**	**multiplex PCR**	***cdtB* PCR**
Amplicon obtained	87	101	69
False positive result	0	0	5
False negative result	19	5	32
Total number of *C. jejuni*/*C. coli* identified by PCR		106	

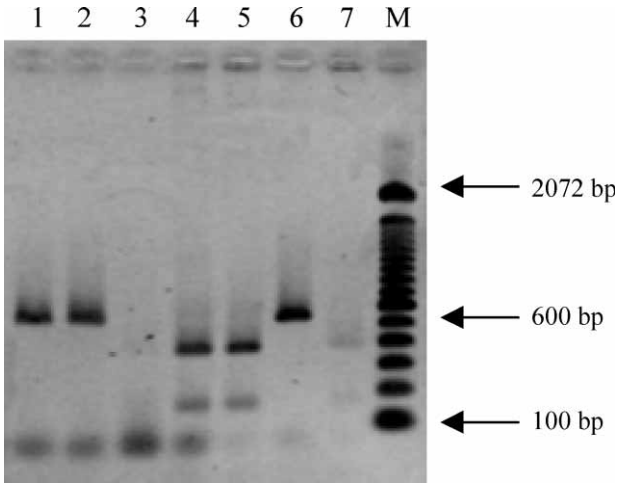

Fig. 3. *Eco*RI restriction digest patterns of the 500 bp PCR product from the amplified *cdtB* gene regions. *CdtB* genes in *C. jejuni* are digested on two fragments of 350 bp and 150 bp (lanes 4, 5 and 7), while *cdtB* genes in *C. coli* remain unrestricted (lanes 1, 2 and 6). M: 100 bp DNA ladder, Gibco.

not clear and no amplicon was obtained for those strains by *cdtB* PCR and multiplex PCR. Other phenotypic characteristics of those strains were specific for *C. jejuni/C. coli*, and they were also positive by *flaA* PCR, so they were considered as *C. jejuni/C. coli.*

Sixty-six (65.3 %) isolates were identified as *C. jejuni* and 35 (34.7 %) isolates as *C. coli*. Thirty-six *C. jejuni* isolates were hippurate negative (Table 3).

We checked the accuracy of *cdtB* identification compared to multiplex PCR. Fifty-eight strains were identified by multiplex PCR and restriction analysis of *cdtB* amplicon and there was 100 % concordance between the results.

Final results of comparative identification of 163 isolates are listed in Table 4.

Discussion

Campylobacter spp. are frequently found in the faeces of healthy animals, especially in birds. Poultry is frequently colonised by thermotolerant *C. jejuni* and *C. coli*. Handling and consumption of undercooked poultry meat is considered to be the source of most human infections with these species. Especially the consumption of chicken liver is a risk factor for human *Campylobacter* enteritis (*3*). The number of human *Campylobacter* infections in Slovenia is increasing. The reasons have not been documented, but they are not the consequence of better diagnostics and/or surveillance system (*13*). There are no data on the extent of poultry meat contamination with this pathogen. For this reason we sampled poultry meat (liver and skin from legs) from 5 main Slovenian suppliers that provide most of the poultry to the retail market. Many of the tested samples (27/33) were positive, which could indicate a high degree of poultry contamination with thermotolerant campylobacters. This is consistent with studies from the USA (*5*), France (*18*) and the UK, but much higher compared to Finland or Denmark (*19*). The extent of contaminated liver and skin samples in our study was similar. Higher degree of contaminated samples was found at samplings in early autumn than in early spring. Samples from all suppliers were contaminated with thermotolerant campylobacters.

Standard method (ISO 10272) for detection of campylobacters in foods is time-consuming, expensive and problematic due to subjective interpretation of the results and the existence of biochemically atypical strains (*10*). For this reason *Campylobacter* is an ideal organism for identification by PCR.

In this study 163 presumptive thermotolerant *Campylobacter* isolates were isolated from the samples using the ISO 10272 method. The species identification was made initially by standardised phenotypic tests and secondly by *flaA* and multiplex PCR. The isolated culture was checked for motility, aerobic growth, growth at 25 °C, the presence of oxidase and catalase, fermentation of

Table 3. Species identification of isolates according to phenotypic identification and two genotypic assays (multiplex PCR and restriction analysis of *cdtB* amplicon)

	Number of isolates	%		%
C. jejuni (hippurate +)	23	22.8		
C. jejuni (hippurate –)	36	35.6	*C. jejuni*	65.3
C. jejuni (hippurate undefined)	7	6.9		
C. coli	35	34.7	*C. coli*	34.7
Total	101	100		100

Table 4. Final results of comparative identification of 163 presumptive thermotolerant *Campylobacter* isolates

Total number of isolates	**163**		
		C. jejuni (hippurat +)	23
		C. jejuni (hippurat –)	36
C.jejuni/C. coli	106	*C. jejuni* (hippurat undefined)	7
		C. coli	35
		C. jejuni/C. coli undefined	5
Thermotolerant campylobacters, but not *C. jejuni/C. coli*	16		
Non-thermotolerant campylobacters	7		
non-*Campylobacter* spp.	34		

sugars and the hydrolysis of the hippurate. For approximately one third of the strains the resistance to antibiotics (cephalothin and nalidixic acid) was also tested, but the interpretation of the results was problematic due to variable resistance zones on the plates (data not shown). The results of these two antibiotic resistance tests are also unreliable for identification of the species due to atypical strains (*7*). For this reason and because of high expenses, those tests were no longer performed.

The standard phenotypic tests for species identification were inadequate since 30.7 % of identifications were either incorrect or unreliable. Ninety-five isolates were initially identified as members of thermotolerant *Campylobacter* species and only 24 of those isolates were hippurate positive. The hippurate hydrolysis test, which is the phenotypic test most commonly used in routine diagnostics, was unable to identify 36 *C. jejuni* isolates in this study. This is consistent with some previous studies (*20,21*). Several mistakes were done in the interpretation of the results of oxidase test (n=8) and test of growth at 25 °C (n=2). Seventeen strains were misidentified because present in a mixed culture. However, clearly positive hippurate reaction always correlated to an isolate genotyped as *C. jejuni*. But, as mentioned earlier, because of the existence of many hippurate negative *C. jejuni* strains, this biochemical test is not reliable for identification of *C. jejuni*.

DNA was isolated by two methods, a classical procedure with the use of guanidinium thiocyanate (*14*) and simple boiling procedure (*15*). The latter was found convenient for the type of analysis we performed. For the isolates where amplification was not successful by using DNA isolated by short procedure, the result was also negative with the DNA isolated in a classical way (*14*).

The comparison between genotypic assays used and phenotypic method was done. Both approaches gave the same result for approximately 70 % of isolates. The remaining 30 % of isolates were positive either by phenotypic or genotypic method, but not with both (Table 1a). Mistakes were done as described above in the interpretation of some phenotypic test results. The presence of generally biochemically inactive *Campylobacter* species was mimicked by contaminants when the culture was not pure. However, in such cases, campylobacters were identified by PCR identification methods, because of their specificity and this is in fact one of the important advantages of these methods.

Final results of comparative identification for 163 isolates tested in our study are shown in Table 4.

The evaluation of applicability of different PCR systems for identifying C. jejuni and C. coli

Flagellin genes are typically present in *C. jejuni* and *C. coli (11*). With amplification of *flaA* gene we identified 87 isolates as *C. jejuni/C. coli*. This approach showed to be reliable, fast and easy to perform. The disadvantage of the assay is that we could not differentiate between the two species. In order to avoid this, the multiplex PCR was used that allowed the differentiation between *C. jejuni* and *C. coli*. This method is able to identify gene encoding hippuricase (*hip*) present in *C. jejuni* and species specific regions in *C. coli* in one single reaction (*17*). In such a way we identified 101 isolates. Eighty-two of them were identified also with *flaA* PCR, but multiplex PCR identified 19 isolates where *flaA* PCR failed. Higher yield, shorter time and ability to differentiate between the two species showed to be the advantages of multiplex PCR compared to *flaA* PCR. Both genotypic systems gave no false positive results, all the positive strains were also confirmed by phenotypic tests (Table 2).

The differentiation between *C. jejuni* and *C. coli* was performed by three methods. We tested the reliability of restriction analysis of *cdtB* amplicon. This method discriminates between the species *C. jejuni* and *C. coli*, because *cdtB* gene in *C. coli* lacks the restriction site for *Eco*RI (*12*). The isolates were considered *C. jejuni* when they were hippurate positive and were also identified as *C. jejuni* by both genotypic systems. Genotypically positive and hippurate negative isolates were considered as hippurate negative *C. jejuni*. The isolates were identified as *C. coli* when so identified with genotypic methods and were hippurate negative. Sixty-six (65.3 %) isolates were identified as *C. jejuni* and 35 (34.7 %) isolates as *C. coli* (Table 3). Although *C. jejuni* was predominating, the extent of *C. coli* was very high according to the published data (*22*). However, such proportion of *C. coli* is in concordance with studies from Bosnia and Herzegovina, where 36 % of *C. coli* strains were found among clinical isolates as well as with reports from Croatia (35 %) (*24,25*) and Central African Republic (39 %). Our study supports the suggestion from Bosnian study that poultry may be the primary source of *C. coli.* These findings could be interesting in connection with already reported higher antibiotic resistance of *C. coli* strains (*26*). The antibiotic resistance of isolates from this study is currently under investigation.

A high proportion (54 %) of *C. jejuni* isolates were hippurate negative. Those isolates were misidentified by phenotypic tests and this shows the unreliability of phenotypic identification.

Identification by restriction analysis of *cdtB* amplicon showed to be reliable, while the identification results were in 100 % concordance with the results of the other two methods. The problem of this method is in amplification of *cdtB* gene, because many false negative results were obtained. Most of the strains (28/32), where *cdtB* genes were not amplified, were identified as *C. jejuni*. *CdtB* PCR unfortunately has not been extensively used, so a comparison of the results is difficult. However, Eyigor *et al.* (*27*) in their study examined 13 *C. jejuni* and 11 *C. coli* poultry meat strains for the presence of *cdtB* gene. It was found in all *C. coli* strains but not in all *C. jejuni* strains (11/13). Those results could indicate that *cdtB* genes lack more often in *C. jejuni* than in *C. coli*, but total number of the tested strains is very low. More investigation is needed to further the current knowledge about the prevalence of *cdtB* genes in isolates from poultry meat and other sources.

In five cases the *cdtB* amplicon was obtained, although with other methods the isolates were identified as non-campylobacters. This could be explained by the reports that *cdt* genes are present also in some other species (*28*). The lower specificity could be also due to non-stringent conditions of the reaction (*12*).

Conclusions

High extent of *Campylobacter* contamination of poultry meat was found with a significant proportion of *C. coli*. The use of traditional phenotypic tests to identify *C. jejuni* and *C. coli* was found to be inconvenient, because of possible misinterpretation of test results and because of *C. jejuni* strains with atypical phenotypes. Of all tested PCR assays, multiplex PCR, targeting hippuricase gene in *C. jejuni* and species specific region in *C. coli*, was the most efficient because fast identification of *C. jejuni* and *C. coli* without further analysis was allowed. *FlaA* PCR showed to be a convenient method to identify the group *C. jejuni*/*C. coli*, but it does not differentiate between the two species. *CdtB* PCR is inconvenient because of many false negative and some false positive results.

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Klasična i molekularna identifikacija termotolerantnih kampilobaktera iz piletine

Sažetak

Ispitana je prisutnost termotolerantnih bakterija roda *Campylobacter* u uzorcima piletine sa slovenskog tržišta. Izolirani su sojevi identificirani fenotipskim i genotipskim postupcima. Fenotipska je identifikacija provedena klasičnim postupkom ISO 10272. Da bi se identificirali određeni sojevi, ispitana je primjenjivost različitih PCR postupaka, korištenjem specifičnih DNA regija za vrste *C. jejuni* i *C. coli*. Veliki broj ispitivanih uzoraka bio je pozitivan (27/33), sa značajnim udjelom *C. coli* u tim dvama identificiranim sojevima (32 %). Veliki udjel sojeva *C. jejuni* (54 %) bio je hipurat negativan. Fenotipska je identifikacija stoga neprikladan postupak zbog prisutnosti sojeva s atipičnim fenotipom, a i zbog mo-

gućnosti pogrešne interpretacije rezultata testova. Multipleks PCR-postupak, koristeći hipurikazni gen u *C. jejuni* i u specifičnoj regiji za vrstu *C. coli*, pokazao se uspješnim jer omogućava brzu, jednostavnu i točnu identifikaciju *C. jejuni* i *C. coli*. Postupak PCR s lokusom flagelarnoga gena (*flaA* PCR) pouzdan je postupak za identifikaciju *C. jejuni*/*C. coli*, ali se njime ne mogu razlikovati te dvije vrste. Postupak PCR s lokusom gena citoletalnog toksina (*cdtB* PCR) nije prikladan jer daje puno lažno negativnih, a i nešto lažno pozitivnih rezultata.