MONITORING OF SELECTED GENES IN CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI ISOLATES FROM DOMESTIC ANIMALS

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Abstract

In the present study, the presence of flaA, cadF, cdtB, and iam genes of Campylobacter sp. were analysed using PCR. Material for analyses comprised 100 Campylobacter sp. isolates obtained from healthy broiler chickens, fatteners, and calves, among which 84 isolates were ascribed to Campylobacter jejuni and 16 to Campylobacter coli. All isolates (100%) had the cadF gene responsible for adhesion and the flaA gene determining the motility of the analysed bacteria. The frequency of occurrence of the cdtB gene responsible for the production of the cytolethal distending toxin (CDT) was determined to be high (98.6% in broiler chickens, 75% in fatteners, 62.5% in calves). In case of the iam gene, the highest frequency was recorded in Campylobacter sp. isolated from broiler chickens (84.7%), while in strains collected from fatteners and calves it was lower, amounting to 41.7% and 18.8%, respectively.

Key words: domestic animals, Campylobacter jejuni, Campylobacter coli, genes.

Natural reservoirs of bacteria from the genus Campylobacter are found in the alimentary tract of birds, dogs, cats, and other animals, where they constitute the components of the commensal flora. They may also be isolated from the natural environment, e.g. the surface of water bodies (1). The genus Campylobacter is represented by over two hundred species and subspecies, including the frequently isolated Campylobacter jejuni and Campylobacter coli. Campylobacter sp. are the most commonly isolated etiological factors in food poisonings in animals and humans. Infection occurs through human contact with infected poultry meat, water, or unpasteurised milk (11). Factors determining pathogenicity of Campylobacter sp. include motility and chemotaxis, as well as adhesion and invasiveness (15). At present, it is believed that selected genes are responsible for pathogenicity of Campylobacter sp., i.e. the flaA gene determining motility, cadF affecting adhesion, cdtB responsible for toxin production, and iam determining invasiveness (9). The flaA gene encodes protein FlaA, which is present in the cilia and influences the cillum length. The cadF gene influences adhesin production in the outer membranes of bacteria, e.g. protein CadF, which binds fibronectin in enterocytes (5). The cdtB gene is responsible for the production of proteins exhibiting toxic properties, i.e. cytolethal distending toxin (CDT) that affects DNA degradation in the host (13). Differentiation of Campylobacter jejuni and Campylobacter coli based only on biochemical tests does not seem completely reliable, after hipurane-negative strains have been detected. The PCR method with the application of primers complementary to the sequences of rRNA coding the abovementioned genes proves to be more accurate.

The aim of this study was to show the varied shares of virulence genetic markers among strains isolated from healthy pigs, chickens, and calves. This investigation may contribute in the future to the determination of a correlation between evoked disease symptoms and the genotype of isolated strains of Campylobacter sp.

Material and Methods

A total of 100 Campylobacter sp. strains isolated from healthy pigs, chickens, and calves were used in this study. Seventy-two strains were isolated from the rectum of broilers, 12 from porkers, and 16 from calves. Campylobacter isolates were cultured at 42°C ±1 in Campy Selective Agar Base Preston (Neogen) for 48 h in an atmosphere composed of 6% oxygen, 10% carbon dioxide, and 84% nitrogen.
Campylobacter sp. identification was performed using multiplex PCR for the simultaneous detection of Campylobacter jejuni and Campylobacter coli. The following reference strains: C. jejuni ATCC 33560 and C. coli ATCC 33559 were also included. All strains were preserved in 20% glycerol at -70°C.

Extraction of DNA (1) was performed using CHELEX-100 chelating resin (Bio-Rad). Bacterial colonies were suspended in 100 µl of TRIS and 45 µl of 20% CHELEX and boiled for 10 min. Samples were then immediately placed on ice for 1 min and centrifuged at 13,000 g for 10 min at room temperature. The supernatant (2 µl) was used in PCR. The purity and concentration of DNA were estimated using spectrophotometry at 260 and 280 nm.

The presence of the cadF, flaA, cdtB, and iam genes was determined with the primers listed in Table 1. All PCR amplifications (1) were performed in a mixture (25 µl) containing: 2.5 µl of the PCR buffer (10x concentrated), 2.5 µl of MgCl₂ (25 mM), 0.5 µl of dNTPs (10 mM), 1 µl of each primer (100 µM), 0.5 µl (1U) of the Taq termstable DNA polymerase (Promega Corporation), 2 µl of the bacterial template DNA, and 15 µl of nuclease free water. The PCR products were analysed by electrophoresis in 1.5% agarose gel. The size of the PCR amplicons was compared to the 100 bp DNA marker (Promega Corporation).

### Results

Table 2 and Fig. 1 present the numbers and percentages of detected genes in Campylobacter sp. strains isolated from different farm animals. The quantitatively predominant strain in the tested animals was C. jejuni in broiler chickens and calves, and C. coli in fatteners.

All isolates of Campylobacter sp. coming from broiler chickens, fatteners, and calves had the cadF gene responsible for adhesion and the flaA gene indirectly affecting the motility in the analysed strains. In case of the cdtB gene, which determines the formation of CDT, the frequency of incidence varied. The cdtB gene was detected in 98.6% of Campylobacter sp. strains, and in 100% of C. jejuni and in 75% of C. coli strains. Strains of Campylobacter sp. collected from fatteners contained the cdtB gene in 75% cases. The cdtB gene was identified in 100% isolates of C. jejuni and in 66.7% isolates of C. coli.

### Table 1

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’→3’)</th>
<th>Product bp</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cadF-F</td>
<td>TGGAGGTTAATTAGATATGG</td>
<td>400</td>
<td>(8)</td>
</tr>
<tr>
<td>cadF-R</td>
<td>CTAACCTTAAAGTGAAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flaA-F</td>
<td>GATTTCGTATTAACAAATGGTG</td>
<td>1728</td>
<td>(12)</td>
</tr>
<tr>
<td>flaA-R</td>
<td>CTGTAGTAACTTAAAAACATTIT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cdtB-F</td>
<td>GTAAAATCTCTGCTATCAACCA</td>
<td>495</td>
<td>(2)</td>
</tr>
<tr>
<td>cdtB-R</td>
<td>GTGGGCACITTGGAATTGCAAGGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iam-F</td>
<td>GGCAGAAAATATTATCACCC</td>
<td>518</td>
<td>(3)</td>
</tr>
<tr>
<td>iam-R</td>
<td>TTCAGCTACTATGCGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F – forward primers, R – reverse primers

In calves, the cdtB gene was found in 62.5% of the analysed isolates of Campylobacter sp. The cdtB gene was detected in 100% isolates of C. coli and in 53.8% isolates of C. jejuni.

The frequency of incidence of the iam gene, determining invasiveness in the analysed bacteria, also varied. In case of broiler chickens, the iam gene was isolated from 84.7% of Campylobacter sp. isolates, among which it was found in 88.2% of C. jejuni and in 25% of C. coli strains.

Isolates of Campylobacteri sp. collected from fatteners had the iam gene in 41.7% of cases. In C. jejuni this gene was identified in 33.3% of the isolates, while in C. coli in 44.4% of the isolates. The lowest percentage of incidence of the iam gene was recorded for isolates collected from calves. This gene was found in 18.8% of strains, of which it was identified in 33.3% of C. coli and in 15.5% of C. jejuni.
Discussion

Campylobacteriosis has been the most frequently reported human food-borne zoonosis in the EU since 2004 (4). Campylobacter sp. is capable of colonising the alimentary tract of all animal species. It is most frequently isolated in fowl. In gallinaceous poultry, C. jejuni is the dominant species (approx. 90% isolations), while C. coli is found less frequently. According to the EFSA report (4), the risk of campylobacteriosis in humans is associated with handling, preparation, and consumption of broiler meat. The occurrence of C. jejuni/coli in wild fowl varies and ranges from 0% to 80% (17). The species isolated most frequently from the alimentary tract of cattle is also C. jejuni, whereas C. coli is identified less commonly. They are isolated much more frequently from young animals than older cattle but their role as the main cause of diarrhoea is rarely observed (9). In pigs, C. coli is a frequently isolated species. It is found as a component of the normal intestinal flora; however, it may cause lesions in the small intestine and duodenum (14).

Studies conducted to date show a diverse share of virulence genetic markers and differences in genotypes of Campylobacter sp. strains isolated from animals and humans. An important factor in virulence of Campylobacter is its motility and chemotaxis. Cilium filaments in C. jejuni contain two cilia proteins: FlaA and FlaB encoded by genes flaA and flaB, which are responsible for motility and virulence of these bacteria (9, 19). In this study, the presence of the flaA gene was observed in all the analysed isolates. Upon reaching the intestine, strains of Campylobacter sp. attach to the intestinal epithelium and penetrate it. At this stage interactions may occur with epithelial cells and proteins produced by the bacteria, leading to absorption disorders in the intestines.

Another virulence agent is the cadF gene responsible for the production of adhesin proteins, outer membrane proteins, i.e. PEB1, JlpA, and CadF, as well as glycolipids (7). In the opinion of many authors the cadF gene is necessary for Campylobacter sp. to cause campylobacteriosis in humans (18). However, it was found that mutants of CadF are not capable of colonising intestines e.g. in newly hatched chickens (20).

In this study, the iam gene, responsible for invasiveness, was found in Campylobacter sp. strains at a variable level. Carvalho et al. (3) observed that the iam gene was detected most frequently in strains of C. jejuni rather than C. coli, but it may also be closely connected with adhesion in those bacteria.

All the analysed strains of C. jejuni and C. coli carried the cdtB gene encoding a protein exhibiting toxic properties, i.e. cytolethal distending toxin (CDT). This exotoxin causes the inhibition of the cell cycle and DNA degradation in the host (9) and may cause death of sensitive eukaryotic cells (6). CDT is composed of three subunits CdtA, CdtB, and CdtC encoded by cdtA, cdtB, and cdtC genes. All the three subunits are required for full activity (16).

Summing up, it should be underlined that Campylobacter sp. may cause a greater number of alimentary tract infections in animals and humans in comparison to bacteria from the genus Salmonella. Analyzed animals were asymptomatic carriers of these rods. However, a considerable distribution of the cadF and flaA genes, as well as the lesser incidence of cdtB and iam genes in strains isolated from these animals may pose a serious threat connected with an increase in their pathogenicity.

References


