DNA Fingerprinting and Serotyping of *Campylobacter jejuni*
Isolates from Epidemic Outbreaks

LENALIND,1* EVA SJÖGRENN,1 KJETILMELBY,2 ANDBERTIKAIJSER1

Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden,1 and
Department of Microbiology, Ullevaal University Hospital, Oslo, Norway2

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The aim of the present investigation was to compare DNA fingerprinting and serotyping (heat-stable and heat-labile antigens) of isolates from epidemic outbreaks as well as of solitary isolates. *Campylobacter jejuni* isolates from two epidemic outbreaks in Sweden, one milkborne (35 isolates) and one waterborne (17 isolates), and one waterborne outbreak in Norway (11 isolates), as well as 30 solitary isolates from Swedish patients with gastroenteritis, were analyzed. A total of 93 isolates were analyzed. In the waterborne outbreak in Norway, only one serotype with one DNA pattern was found. In the milkborne outbreak in Sweden, two serotypes (HS2:HL4 and HSNT:HL4) with two different DNA patterns were found. The isolates from the waterborne outbreak in Sweden were different serotypes. For two isolates of the same serotype, different DNA patterns were seen. This was also recorded for isolates from solitary cases. It was concluded that serotyping is a useful tool in most epidemiological situations but sometimes lacks sufficient discriminatory power. DNA fingerprinting can add valuable epidemiological information to that supplied by serotyping and can in some situations provide sufficient epidemiological information when used alone.

*Campylobacter jejuni* is a commensal organism normally found in the wild as well as in domestic animals (6, 23). Furthermore, it is found at a fairly high frequency in river water and ponds, during both warm and cold seasons (2, 24). Most persons infected with *C. jejuni* have solitary infections originating from contaminated food. Secondary cases are uncommon (1, 3, 8).

Various species of *Campylobacter* have been known for 15 years to have an important role in human gastrointestinal infections. Originally, two species were described, *C. jejuni* and *C. coli*. Thereafter, more species with various degrees of clinical significance were recognized, some pathogenic and some nonpathogenic. In the mid-1980s, *Campylobacter pylori* was recognized as a specific genus and was named Helicobacter. Many *Campylobacter* species cause diarrhea. *Helicobacter* spp. contribute to the etiology of gastritis and ulcers.

*C. jejuni* is one of the most common bacterial etiologies of waterborne outbreaks of diarrhea (12, 14, 25). One explanation is that *C. jejuni* may survive under different conditions in a nonculturable form (7, 15, 21).

Several waterborne epidemics have previously been described. Three well-known and well-documented epidemics occurred in Vermont in 1982 (25), in Grums, Sweden, in 1986 (14), and in northern Norway in 1988 (12). The outbreaks often are small, affecting a limited number of individuals. Those mentioned above, however, involved hundreds of people.

In addition to water, unpasteurized milk has been recognized as a source of epidemic outbreaks of diarrhea (22). Approximately 5% of cattle (23) have *Campylobacter* spp. as a component of the gut flora. We have recently studied three epidemic outbreaks. One waterborne outbreak occurred in northern Norway, originating from contaminated drinking water. It was concluded that the source of the organism was the polluted surface water of a small lake which is used for drinking water in that part of Norway. The contamination possibly came from feces of wild birds in the surrounding area. The second epidemic outbreak was in Sweden, and the organisms originated from a small river, where the river water via backflow contaminated the drinking water system. Organisms of the third small outbreak originated from infected cows. The owner family drank unpasteurized milk.

In epidemic outbreaks caused by bacteria, there is an urgent need for a rapid, reliable, and simple typing procedure in order to detect and eliminate the source of infection. For *Campylobacter* spp. as well as for many other enteropathogenic microorganisms, a number of methods, such as serotyping, ribotyping, auxotyping, and plasmid typing, have been established (17, 18).

A commonly used serotyping system is based on surface antigens (8). The aim of this investigation was to compare the usefulness of the recently described DNA fingerprinting typing procedure with heat-stable (HS)- and heat-labile (HL)-antigen serotyping techniques using isolates from singly infected persons as well as isolates from epidemic outbreaks (13).

**MATERIALS AND METHODS**

**Bacterial isolates.** Isolates of *C. jejuni* from three epidemic outbreaks were investigated. From the milkborne outbreak, *C. jejuni* isolates from the feces of 30 cows the milk of 1 cow, and the stools of four farmers with diarrhea were investigated. From a waterborne outbreak in Sweden, *C. jejuni* isolates from 13 patients with diarrhea and four water samples were tested. From a waterborne outbreak in Norway, *C. jejuni* isolates from 11 patients with diarrhea were investigated.

Thirty isolates from patients (18 with serotype HS2:HL4, 5 with serotype HS18:HL2, and 7 with serotype HS1:HL2) without any epidemiological connection to an outbreak were also tested.

All isolates were kept freeze-dried until tested.

**Preparation of chromosomal DNA from plate cultures.** Freeze-dried isolates of *Campylobacter* were mixed with 1 ml of sterile broth and plated on Columbia blood agar containing 5% horse blood. The plates were incubated overnight at 42°C under microaerophilic conditions (5% O2, 10% CO2, and 85% N2). The *Campylobacter* cells were scraped off and resuspended in 1.5 ml of 0.9% NaCl in an Eppendorf tube and spun down at 6,000 × g for 5 min. The pellet was resuspended in 540 μl of 0.01 M Tris-EDTA (TE) buffer (pH 8.0) containing 5 mg of lysozyme (Sigma) per ml. A 60-μl volume of 10% sodium dodecyl sulfate
DNAs from three isolates of *C. jejuni* were digested with either *Apa*I or *Nol*I; DNAs from 18 isolates were digested with either *Hin*dIII, *Bam*HI, or *Bst*36 I; DNAs from 93 isolates were digested with *Hae*III; and DNAs from 55 isolates were digested with *Pst*I (see Table 1) (4, 5, 9, 10, 16). All enzymes were obtained from Scandinavian Diagnostic Service.

Electrophoresis and photography. The digested bacterial DNA was loaded on a horizontal slab gel (0.7%) covered with electrophoresis buffer (40 mM Tris acetate [pH 7.9], 2 mM sodium EDTA). The gel was run overnight at 40 V. After electrophoresis, the gel was stained for 45 min with ethidium bromide (0.5 μg/ml) and photographed with UV illumination and Polaroid film (Polaroid 667).

Serotyping. The 93 *C. jejuni* isolates included were serotyped for HS (19, 20) and HL antigens (11). A total of 65 different absorbed antisera were used for HS typing, and 125 absorbed antiserum were used for HL typing. The absorption was performed as described by Penner et al. (19) and Lior et al. (11), respectively.

**FIG. 1.** DNA fingerprinting profiles II to VIII of the isolates from the Swedish waterborne outbreak. Lanes 1, 2, and 4, DNA pattern II; lanes 3, 8 to 12, and 14 to 16, pattern III; lane 5, pattern IV; lane 6, pattern V; lane 7, pattern VI; lane 13, pattern VII; and lane 17, pattern VIII.

**FIG. 2.** DNA fingerprinting profiles I of the 11 isolates (lanes 1 to 11) from the Norwegian waterborne outbreak.

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### TABLE 1. Testing of bacterial strains with different restriction endonucleases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of strains tested</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apa</em>I</td>
<td>3</td>
<td>Not digested</td>
</tr>
<tr>
<td><em>Nol</em>I</td>
<td>3</td>
<td>Not digested</td>
</tr>
<tr>
<td><em>Hind</em>III</td>
<td>18</td>
<td>Partly digested</td>
</tr>
<tr>
<td><em>Bam</em>HI</td>
<td>18</td>
<td>Partly digested</td>
</tr>
<tr>
<td><em>Bst</em>36 I</td>
<td>18</td>
<td>No distinct pattern when DNA fragments were &gt;10 kb</td>
</tr>
<tr>
<td><em>Hae</em>III</td>
<td>93</td>
<td>Distinct pattern</td>
</tr>
<tr>
<td><em>Pst</em>I</td>
<td>55</td>
<td>No distinct pattern when DNA fragments were &gt;10 kb</td>
</tr>
</tbody>
</table>

(SDS) was added, and the solution was incubated at 37°C for 15 min. One hundred fifty microliters of a solution containing 25 mg of pronase (Boehringer Mannheim GmbH) per ml was added, and the combination was mixed and reincubated overnight at 37°C and then centrifuged at 10,000 × g for 15 min. Thereafter, 700 μl of the supernatant was transferred to another Eppendorf tube (4). For DNA extraction, 700 μl of phenol was added and the mixture was vigorously shaken and then centrifuged at 10,000 × g for 5 min. Approximately 75% of the viscous supernatant above the white interface was transferred to a fresh Eppendorf tube, an equal volume of chloroform-isooamyl alcohol was added, and the mixture was carefully combined and centrifuged at 10,000 × g for 2 min. The supernatant was once more mixed with an equal volume of chloroform-isooamyl alcohol and centrifuged as described above, and the supernatant was again transferred to a fresh Eppendorf tube. The supernatant was made 1 M by adding 5 M NaCl, and the DNA was precipitated with 2 volumes of 96% ethanol for 30 min at −20°C and centrifuged at 10,000 × g for 10 min. The pellet, which consisted of DNA, was suspended in 100 μl of TE buffer (pH 7.6) and treated with 0.5 μl of a solution containing 10 mg of RNase (Boehringer Mannheim GmbH) per ml for 45 min at 37°C. A 50-μl volume of 7.5 M NH₄ acetate was added, and the DNA was precipitated with 375 μl of 96% ethanol for 30 min at −20°C and centrifuged at 10,000 × g for 10 min. The pellet was resuspended in 100 μl of TE buffer (pH 7.6). The final DNA concentration was measured spectrophotometrically (GeneQuant II; Pharmacia Biotech) at 260 nm (5).

Restriction endonuclease digestion of DNA. Different restriction endonucleases were examined to find out which one gave the most distinct DNA pattern.

### TABLE 2. Campylobacter isolates, serotypes, and *Hae*III restriction endonuclease digest DNA patterns from epidemic outbreaks and from solitary patients with diarrhea

<table>
<thead>
<tr>
<th>Origin and source of specimen</th>
<th>No. of isolates</th>
<th>Serotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DNA pattern(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterborne outbreak, Norway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>11</td>
<td>HS7:HL5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>I</td>
</tr>
<tr>
<td>Waterborne outbreak, Sweden</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>3</td>
<td>HSNT:HL55</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>HS23:HL7</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>HS1,44:HL10,13</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>HS23:HLNT</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>HS1,44:HL10,13</td>
<td>VI</td>
</tr>
<tr>
<td>Water</td>
<td>2</td>
<td>HS23:HL7</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>HSNT:HL51</td>
<td>VII</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>HS22,23:HL13</td>
<td>VIII</td>
</tr>
<tr>
<td>Milkborne outbreak, Sweden</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>4</td>
<td>HS2:HL4</td>
<td>IX</td>
</tr>
<tr>
<td>Cow milk</td>
<td>1</td>
<td>HS2:HL4</td>
<td>IX</td>
</tr>
<tr>
<td>Cow feces</td>
<td>29</td>
<td>HS2:HL4</td>
<td>IX</td>
</tr>
<tr>
<td>Solitary patients with diarrhea</td>
<td>18</td>
<td>HS2:HL4</td>
<td>XI–XVIII</td>
</tr>
<tr>
<td></td>
<td>5HS18:HL2</td>
<td>XIX–XXII</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>HS1:HL2</td>
<td>XXIII–XXVII</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> NT, not typeable.
RESULTS

Neither ApaI nor NotI digested the DNA at all. With either HindIII or BamHI, the DNA was only partly digested. Digestion with either Bsu 361 or PstI gave no distinct DNA pattern when the DNA fragments were >10 kb. HaeIII digestion gave the best-resolved pattern even when the DNA fragments were >10 kb, so DNAs from all isolates were digested with HaeIII (Table 1).

Eleven isolates from the Norwegian waterborne outbreak were all identified as serotype HS7:HL5. These isolates also had the same DNA pattern (Table 2 and Fig. 1).

The results for isolates from the waterborne outbreak in Sweden were more complex. Six serotypes and seven DNA patterns were identified among the 17 C. jejuni isolates (Table 2 and Fig. 2). For two serotypes, HS23:HL7 and HS1,44:HL10,13, two different DNA patterns were found.

For the 35 isolates from the milkborne outbreak in Sweden, two serotypes, HS2:HL4 and HSNT:HL4, were identified among the specimens from farmers, cow milk, and cow feces. Thirty-four isolates had the same DNA pattern. For the isolate from the feces of a single cow, with the serotype HSNT:HL4, a different DNA pattern was also found (Table 2 and Fig. 4).

In the group of 30 isolates with no epidemiological connection, we found eight different DNA patterns among the 18 isolates of serotype HS2:HL4, four DNA patterns among five isolates of serotype HS18:HL2, and five DNA patterns among the seven isolates of serotype HS1:HL2 (Table 2 and Fig. 3 through 6).

Among the 93 isolates that were tested, a total of 27 DNA patterns were identified.

DISCUSSION

In order to find a wide spectrum of epidemiologically different isolates, we selected C. jejuni isolates from solitary infected individuals as well as isolates from epidemic outbreaks.
Enteropathogens other than *C. jejuni* were also recovered from fecal samples from the infected patients. The origin of this outbreak was identified as wastewater contamination of the community drinking water pipeline system. Several different strains might therefore have caused the outbreak.

For the solitary isolates from patients with no epidemiological connection, several DNA patterns could also be identified within each serotype (Table 2; Fig. 4 through 6).

Thus, for a number of our isolated serotypes, several DNA patterns were found. However, from a reversed perspective, for each DNA pattern never more than one serotype was found.

We conclude that serotyping is a useful tool in most epidemiological situations and easy to perform. In some of the isolates with one serotype pattern, several different DNA patterns were found. Thus, DNA fingerprinting adds to the epidemiological information supplied by serotyping. In laboratories in which DNA fingerprinting but not *Campylobacter* serotyping is available, the DNA technique may, in the same instance, supply sufficient information for a limited epidemiological investigation.

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**REFERENCES**


