Application of Lior Biotyping by Use of Genetically Identified Campylobacter Strains

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We used the scheme of Lior to biotype 140 genetically identified Campylobacter strains. Our results confirmed previous studies and extended Lior biotyping to show that nine C. jejuni subsp. doylei strains (100%) were one biotype and nine C. jejuni subsp. jejuni nalidixic acid-resistant strains (100%) were C. jejuni biotype I or II. All C. jejuni subsp. jejuni hippurate-negative strains studied and 6 of 35 C. lari strains (17%) were grouped with C. coli biotypes. These findings may be useful in epidemiologic investigations.

Surveillance of Campylobacter species, the most common cause of human bacterial gastroenteritis, depends largely on subtyping procedures that detect unique differences within the strains of a species. Both phenotypic and genotypic methods have been useful for subtyping these bacteria, and because of their ease in performance, phenotypic methods such as serotyping and biotyping are most widely used (12, 13). Several biotyping schemes to distinguish strains of C. jejuni, C. coli, and C. lari, commonly called the thermophilic campylobacters, have been described (2, 6, 12, 14). Except for the Preston scheme (2), biotyping does not identify strains to species level. Identification to species level is carried out by more extensive biochemical and growth tests, confirmed in some cases by cellular fatty acid profiles and more recently by genetic methods. Because strain identity is necessary, it is fundamental that species be known prior to biotyping Campylobacter strains (7). We biotyped 140 Campylobacter strains, with species identity determined by DNA homology and/or multilocus enzyme electrophoresis as described by Lior (6). The Lior scheme separates C. jejuni, C. coli, and C. lari isolates into eight biotypes by the ninhydrin hippurate hydrolysis, DNA hydrolysis, and rapid H2S procedures.

The test strains were 37 hippurate-positive C. jejuni subsp. jejuni isolates, 11 hippurate-negative C. jejuni subsp. jejuni isolates, 9 nalidixic acid (NA)-resistant C. jejuni subsp. jejuni isolates, 9 C. jejuni subsp. doylei isolates, 39 C. coli isolates, and 35 C. lari isolates. They included 99 strains from humans, 38 strains from nonhuman sources, and 3 strains of unknown origin (Table 1) from nine countries. All strains were coded and grown on heart infusion agar with 5% rabbit blood (HIA-Rb) at 36°C for 24 h in a microaerobic atmosphere of approximately 5% O2, 7.5% CO2, 7.5% H2, and 80% N2. Each strain was tested two or more times by each procedure.

We tested for hydrolysis of hippurate by a modified Hwang and Ederer method described by Morris and Patton (10). A deep purple color was a positive reaction. The rapid H2S test (6) required a semisolid medium that was freshly prepared every 2 weeks. The basal component of the medium (Albimi brucella broth; GIBCO, Burlington, Ontario, Canada) is no longer commercially available. We prepared the Albimi formulation of brucella broth by adding sodium citrate (0.1 g/100 ml of broth) to BBL brucella broth (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The type strains of C. jejuni, C. coli, and C. lari from the American Type Culture Collection gave the same H2S results with the commercial and our laboratory-prepared Albimi media. Growth from a 24-h culture on HIA-Rb was rolled into a bead, gently suspended in the upper third of a tube of the semisolid medium, and incubated for 2 h in a 37°C water bath. A positive test showed black discoloration around the bacterial pellet, whereas negative tests had no color change. For the DNA hydrolysis test, a loopful of a

**TABLE 1.** Biological origins of *Campylobacter* test strains

<table>
<thead>
<tr>
<th>Campylobacter speciesa</th>
<th>Nonhuman</th>
<th>Human</th>
<th>Water</th>
<th>Sheep</th>
<th>Bird</th>
<th>Goat</th>
<th>Poultry</th>
<th>Cow</th>
<th>Unknown</th>
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</thead>
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</tr>
<tr>
<td>C. jejuni subsp. jejuni, hipp.&quot;</td>
<td>27</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. jejuni subsp. jejuni, NA resistant</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. jejuni subsp. doylei</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>C. jejuni subsp. jejuni, hipp.</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C. coli</td>
<td>26</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>C. lari</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

a Hipp." and hipp., hippurate hydrolysis positive and negative, respectively.

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24-h culture grown at 36°C was spread heavily in a 5-mm-diameter circle on the surface of a toluidine blue-DNA agar plate (8), and the plate was incubated at 42°C under microaerobic conditions. After 48 h, the Campylobacter strains capable of DNA hydrolysis showed clear, pink zones around the inoculum. Negative strains showed either a clear, colorless zone or the absence of a peripheral zone around the inoculum.

Our results were in agreement with those of Lior (6), who identified four biotypes of hippurate-positive C. jejuni, two biotypes of C. coli, and, in a later study, two biotypes of C. lari (7). Typical (hippurate-positive) C. jejuni subsp. jejuni strains in our study were identified as belonging to all four C. jejuni biotypes (Table 2), with 89% of strains (33 of 37) being biotype I or II. The NA-resistant C. jejuni subsp. jejuni strains were divided among C. jejuni biotypes I (56%) and II (44%). Although NA-resistant C. jejuni (52 of 1,407) and C. coli (7 of 393) strains were included in Lior’s initial study (6), these strains were not differentiated from the NA-sensitive strains. C. jejuni strains exhibiting NA resistance have been genetically defined as C. jejuni strains (4). NA resistance among both C. jejuni and C. coli strains is most likely acquired and believed to be chromosome related (16).

Only one biotype was identified among the C. jejuni subsp. doylei strains (Table 2), although DNA digest analysis and electrophoretic protein typing (5, 11) recognize genomic differences among strains of this subspecies. Neither Lior biotyping study included C. jejuni subsp. doylei strains (6, 7). Our results suggest that additional tests are needed to improve discrimination of C. jejuni subsp. doylei strains to make biotyping applicable to epidemiologic studies. C. jejuni subsp. doylei strains have been isolated from children with diarrhea and from adults with gastric ulcers and chronic gastritis, but their pathogenicity is not known (15).

Biotypes of the hippurate-negative test strains are also listed in Table 2. The C. coli strains were C. coli biotypes I (67%) and II (33%). The hippurate-negative C. jejuni strains, likewise, were grouped with C. coli biotypes I and II in approximately the same ratio (64 and 34%, respectively). Of the 35 C. lari strains, 29 (83%) were C. lari biotype I or II and 6 strains (17%) were grouped with C. coli biotype I. The C. lari isolates that were grouped with C. coli biotype I isolates differed from the other C. lari biotypes by being rapid H2S test negative. To our knowledge, this is the first report of rapid H2S test-negative strains among the classical (typical) C. lari strains. Variants of C. lari, which differ phenotypically in numerous aspects (H2S negative, urease positive, and NA sensitive), are found in humans (9) and the environment (3). Although resistance to NA is recognized as an inherent phenotypic trait in C. lari that distinguishes this species from C. jejuni and C. coli (1), variation in NA sensitivity is found in all three species of thermophilic campylobacters (6, 9), making strain identification difficult. Assignment of strains of one species to the biotype of another species, for example, assignment of hippurate-negative C. jejuni isolates to C. coli biotypes I and II and rapid H2S test-negative C. lari isolates to C. coli biotype I (Table 2), is confusing and points out the need for additional tests to biotype variant strains.

Biotyping did not differentiate the human from the nonhuman strains (Table 2) or separate them geographically (data not shown). Our study supports work by others that indicates that birds and animals harbor Campylobacter biotypes similar to those found among humans (6), thus reiterating the mode of zoonotic transmission of this illness. These findings may be useful in epidemiologic investigations of Campylobacter illnesses, particularly when biotyping is used in combination with serotyping or other typing schemes (2, 6).

### REFERENCES


