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Development and Application of a New Scheme for Typing *Campylobacter jejuni* and *Campylobacter coli* by PCR-Based Restriction Fragment Length Polymorphism Analysis

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A molecular typing approach for *Campylobacter jejuni* and *Campylobacter coli* was developed with restriction fragment length polymorphism analysis of a 9.6-kb PCR-amplified portion of the lipopolysaccharide gene cluster. Sixty-one Penner serotype reference strains were analyzed with this new genotyping scheme, and 32 genogroups were found. Eleven additional genogroups were obtained from 87 clinical *C. jejuni* strains tested. This molecular typing method shows a correlation with the Penner heat-stable serotyping method, a phenotypic typing method based on lipopolysaccharide structures that is often used as a "gold standard" for subtyping *Campylobacter* spp. This strong correlation suggests that the data obtained can be directly compared with epidemiological data collected in the past by classical serotyping of *C. jejuni* and *C. coli*. In contrast to the high percentage of nontypeability by phenotyping, this molecular typing method results in 100% typeability and provides a superior alternative to serotyping.

Campylobacter jejuni and *Campylobacter coli* are recognized as two of the most common causes of food-borne bacterial gastroenteritis. Furthermore, *C. jejuni* has been implicated as a frequent antecedent to the development of the neurologic diseases Guillain-Barré syndrome (GBS) (20) and Miller Fisher syndrome (37).

Numerous subtyping methods have been developed to differentiate *Campylobacter* strains for epidemiologic purposes in the past two decades. More than 30 current typing methods have been reviewed elsewhere (27, 28, 29, 38). The various typing systems can be placed in two categories: phenotypic methods, which are based on expressed features such as somatic antigens or enzymatic activity, and genotypic methods, which are based on specific molecular features of chromosomal or plasmid DNA.

Two serotyping schemes have been used exclusively for phenotypic typing in the past, the scheme developed by Penner and Hennessy, which detects heat-stable (HS) antigens (31), and the one developed by Lior et al., which detects heat-labile antigens (16). The former is the most widely accepted and well evaluated phenotypic method. The molecular basis for the HS antigenic diversity in *C. jejuni* and *C. coli* is the expression of somatic (O) lipopolysaccharide (LPS) (17, 18, 21, 22, 33, 34, 35). LPS is a major constituent of the outer membrane in gram-negative bacteria and comprises three covalently linked regions: lipid A, core oligosaccharide (inner core and outer core), and O polysaccharide. The variability of the *Campylobacter* LPS outer core and O polysaccharide is thought to contribute to the antigenic basis of the Penner serotyping system. Serotyping methods like these are time consuming and technically demanding, and antisera are costly to produce, which limits the use of these typing systems to specialized diagnostic laboratories. Furthermore, phenotypes can be unstable, resulting in nonreproducible results or nontypeable strains as well as antiserum cross-reactivity, which hampers the interpretation. Genotyping methods are independent of expressed features and are therefore a better alternative for typing. Several genotyping methods have recently been developed, such as pulsed-field gel electrophoresis (11, 12, 40), amplified fragment length polymorphism (5, 15), flagella gene PCR-restriction fragment length polymorphism (PCR-RFLP) (1, 4, 23, 24, 26), ribotyping (6, 25, 36), and random amplified polymorphic DNA analysis (7, 10, 30). These genotyping systems are more generally available and applicable than phenotypic methods. However, most of these techniques still have their own drawbacks, such as less discriminatory power, poor reproducibility, and complex methodology. The preferred method in terms of handling, costs, and time, is RFLP analysis of PCR products. Such a method has been described for the flagellin genes (23, 26). However, when this method was applied, no correlation could be detected between flagellin genotypes and HS serotypes (23). This greatly reduces the application of flagellin genotyping in long-term epidemiological studies. More importantly, none of these methods correlate well with the serotyping scheme used in past decades, so historical epidemiological trends cannot be determined. For these reasons, genotypic subtyping methods have not been widely used in epidemiological practice and remain to be developed and improved.

The LPS biosynthesis gene cluster of *C. jejuni* 81116 has recently been characterized in our laboratory (9). In this study,

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