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MINIREVIEW

Genotyping of *Campylobacter* spp.

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INTRODUCTION

The genus *Campylobacter* comprises a group of closely related gram-negative bacteria which primarily colonize the gastrointestinal tracts of a wide variety of host species. Some of these bacteria are commensals, but many, particularly *Campylobacter jejuni*, and its close relative *Campylobacter coli*, are enteric pathogens of humans and domestic animals. *C. jejuni* and *C. coli* are common causes of human acute bacterial enteritis worldwide (65). Surveys have suggested that in developed regions, such as Europe and the United States, the incidence of disease associated with *Campylobacter* infections is as high as 1% of the population per annum. In Great Britain the incidence of infection continues to rise, and there were 58,000 reported cases of campylobacteriosis in 1998; this far exceeds the number of reported cases of salmonellosis (5). A detailed understanding of the epidemiology of human infections is essential for the control of this disease. It is widely assumed that campylobacteriosis is primarily a food-borne disease. Case control studies (89) have suggested that a major source of human infection is the handling and consumption of contaminated poultry meat. This suggestion is consistent with studies showing that gastrointestinal tracts of birds are commonly colonized by campylobacters. However, other meat-producing animals, including pigs, cattle, and sheep, are also frequently colonized by *Campylobacter* spp. The relative contributions of these and other potential sources, such as domestic pets, wild birds, wild animals, and contaminated water, to human infection are currently not known. Targeted control of food-borne bacterial pathogens generally depends on identification of sources and routes of transmission. However, because *Campylobacter* spp. are ubiquitous in the environment, cases are sporadic for the most part, and outbreaks are rare, source tracing has proved to be difficult. This difficulty has been compounded by a lack of suitable and readily available methods for identifying or typing, and thereby tracing, individual *Campylobacter* strains that cause human infections. Such tools would also be useful for studying the epidemiology of *Campylobacter* spp. in food-producing animals in the farm environment.

The diversity within *C. jejuni* and *C. coli* has been well established and is detectable at both the phenotypic and genotypic levels. To date, the most widely used phenotypic procedure has been serotyping. There are two generally accepted, well-evaluated serotyping schemes. The Penner scheme is based on heat-stable (HS) antigens using a passive hemagglutination technique (77). The Lior scheme is based on using heat-labile (HL) antigens (50) and a bacterial agglutination method. The major disadvantages of both of these techniques are the high number of untypeable strains and the time-consuming and technically demanding requirements of the techniques. Production and quality control of antiserum reagents for serotyping schemes are costly; consequently, these reagents are not widely available. A recently developed scheme (23) based on HS antigens in which modified antibody production and antigen detection techniques are used may be an improvement for routine use, but this scheme does not solve the problem of restricted reagent availability or the problem of the high level of nontypeability. Because of such problems, the value of serotyping techniques for national and global epidemiological studies has been restricted. Thus, there is a well-recognized need for alternative subtyping schemes. Recently, molecular subtyping methods have been developed. The major advantage of genotyping techniques is that potentially they could be universally available. Some of these techniques, like ribotyping, pulsed-field gel electrophoresis (PFGE), and flagellin typing (*fla* typing), are already in use in a number of laboratories (62). In this review we describe the technologies currently available for genotypic subtyping of *Campylobacter* spp., discuss the advantages and problems of each technique, and indicate the current value of each method.

FLAGELLIN TYPING (*FLA* TYPING)

The flagellin gene locus of *C. jejuni* contains two flagellin genes (*flaA* and *flaB*), which are arranged in tandem and are separated by approximately 170 nucleotides. Because both highly conserved and variable regions are present (56), this locus is suitable for restriction fragment length polymorphism (RFLP) analysis of a PCR product. The conserved regions in this locus are also partially conserved in species other than *C. jejuni*. Thus, the primers used to develop a typing scheme for *C. jejuni* may be used to generate similar schemes for related pathogens. Indeed, *fla* typing has proved to be valuable for the majority of *C. coli* strains and some strains of *Campylobacter lari*, *Campylobacter helveticus* (71), and *C. jejuni* subsp. *doylei* (47a).

At least seven *fla* typing procedures have been developed (2, 6, 7, 9, 44, 60, 64) (Fig. 1), and there is considerable variation in the PCR-RFLP procedures; the DNA preparation techniques (49, 60), primer design (59) (Fig. 1), annealing temperatures (7, 71), restriction enzymes used (2, 7, 9, 60, 71), and genotype nomenclature all vary. Most of the primer sets that have been described were specifically designed for amplification of *flaA* sequences. The total lengths of the restriction fragments observed suggest that only one *fla* gene is amplified in most cases. However, weak bands that may arise from the second *fla* gene are sometimes visible (68). In some methods three primers are used for combined or separate amplification of *flaA* and *flaB* (6, 59). Due to the strong conservation be-

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