Evaluation of Current Molecular Approaches for Genotyping of *Campylobacter jejuni* Strains

Monir U. Ahmed, Louise Dunn, and Elena P. Ivanova

Abstract

*Campylobacter jejuni* has been recognized as the most common bacterial cause of gastroenteritis worldwide, in both developed and developing countries, since the late 1970s. A number of genotyping schemes have been developed to identify the sources and route of transmission of these foodborne pathogens so that proper control measures can be developed. In this review, we provide current genotypic schemes developed for *Campylobacter* spp. (particularly *C. jejuni*) over the last decades, along with an evaluation of the strength and weakness of these techniques and their applications.

Introduction

*CAMPYLOBACTER JEJUNI* HAS BEEN RECOGNIZED as the most common cause of bacterial gastroenteritis worldwide since the late 1970s (Blaser, 1997; Friedman et al., 2004; Jain et al., 2008; Senok and Botta, 2009). The species *C. jejuni* consists of two subspecies, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylfi* (Steele and Owen, 1988; Veron and Chatelai, 1973). In this review, only *C. jejuni* subsp. *jejuni* will be considered and from hereafter will be referred to as *C. jejuni*. In spite of the high prevalence of *C. jejuni* infection in humans, knowledge of the pathogenicity of *C. jejuni* strains is still limited. The type of strains associated with disease manifestations in human is unclear, as is the source and route of transmission of most cases of both sporadic and epidemic campylobacteriosis (Schouls et al., 2003). Such information is critical for targeted control of foodborne diseases. The incidence of foodborne illness is increasing worldwide, due to many factors including the demand for more ready-to-eat, processed foods by consumers, which present major challenges to food safety and quality, globalization of food supplies, and the changing epidemiology of foodborne illness (McLean et al., 2010; Mishra and Sinija, 2008). The ability to rapidly and accurately detect the types of strains associated with outbreaks will not only assist in conducting accurate and timely epidemiological investigations to identify the association between the disease outbreak and source, but enable regulatory authorities to undertake control and preventative measures more efficiently and effectively. Increasingly, outbreak investigations are becoming a major public health activity, and there is a greater dependence on subtype-based surveillance networks to detect geographically dispersed outbreaks, to assist in the early detection of contamination sources that otherwise may be difficult to detect. This assists in identifying and addressing gaps in the food safety system to enable the prevention of foodborne illness (Tauxe et al., 2010).

In order to trace the source and to identify the route of transmission, in both global and local epidemiological studies, potent and well-differentiating typing methods are required. Over the last decades, several typing methods have been developed for *C. jejuni* strain differentiation, with phenotyping methods being routinely used for epidemiological studies of *C. jejuni* (Schouls et al., 2003; Shi et al., 2002) (Fig. 1). The most widely employed phenotypic method is the serotyping technique developed by Penner and Hennessy (Shi et al., 2002), which is based on the detection of heat-stable (HS) antigens. Other phenotypic methods include biotyping, phage typing, and antibiogram. However, these methods are also considered expensive and labor intensive (Schouls et al., 2003). Molecular genotyping methods have the potential to overcome these difficulties; hence, a number of molecular genotyping methods have been tested for epidemiological studies of *C. jejuni* (Schouls et al., 2003). The evolution of genotyping methods for *C. jejuni* and their use and performances are summarized in Figure 1 and Table 1.

The genotyping methods currently developed for *C. jejuni* typing can be divided into three groups based on the evaluation of genetic polymorphism: (a) in whole genome; (b) in a single locus of the genome; (c) in multiple loci of the genome. In this review, we assess the available genotyping approaches and evaluate the advantages and disadvantages of their potential application.
Whole Genome–Based Methods

Whole genome–based methods investigate polymorphism throughout the genome and thus reflect most of the polymorphisms in the strains. There are three genome-based methods—pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), and randomly amplified polymorphic DNA (RAPD)—that can be employed for comparative length polymorphism (AFLP), and randomly amplified polymorphism in the strains. There are three genome-based methods—throughout the genome and thus reflect most of the polymorphisms in the strains. There are three genome-based methods—throughout the genome and thus reflect most of the polymorphisms in the strains. There are three genome-based methods—throughout the genome and thus reflect most of the polymorphisms in the strains.

Pulsed-field gel electrophoresis (PFGE)

PFGE (or micro-restriction profiling) was developed by Schwartz and Cantor (1984) to separate large DNA segments of yeast chromosomes in order to create genomic maps and calculate the genome size of organisms. Since then, PFGE has been commonly used to study the molecular epidemiology of infectious pathogens (Goering, 2010; Ribot et al., 2001) such as Escherichia coli, Streptococcus spp., Staphylococcus spp., Neisseria meningitides, Vibrio cholerae, Bordetella pertussis, and C. jejuni. It is generally accepted as one of the most discriminatory genotyping methods available for genotyping Campylobacter spp. However, the sensitivity and discriminatory power of PFGE depend on the organism being subtyped and on the range of restriction enzymes, for example, SalI, KpnI, SacII, and BamHI (On et al., 1998) and Smal (Ge et al., 2006; Höök et al., 2005; Rönner et al., 2005; Wilson et al., 2009b)). For instance, one rapid PFGE protocol for C. jejuni was based on the standardized PFGE procedure developed by PulseNet (http://www.cdc.gov/ncidod/dbmd/pulsenet/pulsenet.htm), the national network of public health and food regulatory 18/04/1987 at 10:26 AM.€“ produces multiple bands from the whole genome, there is no under-presentation due to point mutations from the whole genome. Since then, PFGE has been extensively used in epidemiological studies of C. jejuni ranging from outbreak investigation (Heuvelink et al., 2009; Olsen et al., 2001; Pitkanen et al., 2008), persistence of genotypes in a human population (Petersen and Wedderkopp, 2001) or environment (Hakkinen and Hanninen, 2009), diversity exploration of sporadic infection isolates (French et al., 2009; Uzunovic-Kamberovic et al., 2007), and dissemination of antibiotic resistant strains (Bae et al., 2007), to the comparison of genotypes within and between hosts (Acke et al., 2010; Parsons et al., 2009; Rozynek et al., 2010), in humans (Islam et al., 2009), and in retail meats (Ge et al., 2006).

Amplified fragment length polymorphism (AFLP)

AFLP, developed initially by Vos et al. (1995) for plant genomes analysis, has been successfully adapted for bacterial genomic DNA fingerprinting. AFLP for epidemiological analysis of C. jejuni isolates was first evaluated by Duim et al. (1999). Later the method was used to study the persistence of C. jejuni clones in environment and host (Fitzgerald et al., 2001b; Manning et al., 2003), genetic relatedness (Alter et al., 2005; On et al., 1998; Siemer et al., 2004), and differentiation among species, subspecies, and strains (Fang et al., 2006). It was also used for detection, identification, and comparison of disease markers (Duim et al., 2001; Godschalk et al., 2006) and strain diversity (Coote et al., 2007). As AFLP is based on selective amplification of restriction fragments generated from the whole genome DNA, this technique is useful for the differentiation of genetically related strains (Janssen et al., 1996). As this method produces multiple bands from the whole genome, there is no under-presentation due to point mutations or single locus recombination (Duim et al., 1999).
<table>
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<td>PCR and HRM</td>
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D, discriminatory power; R, reproducibility; T, typeability; RD, restriction digestion; PCR, polymerase chain reaction; GE, gel electrophoresis, RT-PCR, real-time PCR; SE, sequencing; MS, mass spectrometry; SB, Southern blotting.
AFLP is a three-step technique that involves, after complete digestion of the chromosomal DNA with two restriction enzymes (4- and 6-bp recognition sites) and ligation of oligonucleotide adapters, the selective amplification of restriction fragments, and gel analysis of these amplified fragments (Janssen et al., 1996). The discriminatory power of AFLP is determined by the set of restriction enzymes and primers (Duim et al., 1999, 2003; Janssen et al., 1996).

Whole genome analysis combined with automated data acquisition and analysis led to the development of high-resolution AFLP for C. coli and C. jejuni (Saengthongpinit et al., 2010). This technique does not require prior knowledge of genome sequences, as required in PFGE and RAPD-PCR (de Vos et al., 1995), but it does require intact purified double-stranded DNA, specialized equipment, and software (Duim et al., 1999). Being a gel-based method, it shows differences in fingerprints of identical sample and sensitivity, dependent on the restriction enzymes used. These two factors affect reproducibility of AFLP and limit its routine use (Duim et al., 2001). A modified version of AFLP, fluorescently amplified length polymorphism (FAFLP), which incorporates fluorescently labeled primers (HindIII + A and HhaI + A), has overcome this limitation (Desai et al., 2001). This improved AFLP was successfully used for the epidemiological investigation of campylobacters in the food chain (humans, pigs, cattle, poultry, and retail meats) and for the evaluation of the genetic diversity of thermostolerant campylobacters in commercial broiler flocks and broiler farm environments (Messens et al., 2009).

Randomly amplified polymorphic DNA (RAPD)

RAPD is a rapid typing method based on randomly amplified polymorphic DNA. In this method, a single 10-mer primer is used to amplify multiple locations that are randomly distributed throughout the whole genome and produce a spectrum of amplified products characteristic of the template DNA. The amplified fragments are then separated by agarose gel electrophoresis to create a genetic profile for each isolate. As the entire genome becomes accessible to priming and amplification, polymorphisms throughout the genome can be detected (Welsh and McClelland, 1990; Williams et al., 1990).

The RAPD method for genotyping of Campylobacter spp. was developed by Mazurier et al. (1992) using three 10-mer random primers separately. Later, various primers and reaction conditions were also developed for RAPD analysis of Campylobacter spp. RAPD was found to be highly discriminatory in investigating the genetic diversity of C. jejuni and C. coli isolates from different sources (Hilton et al., 1997; Madden et al., 1996; Misawa et al., 2000; Payne et al., 1999). The method is simple and rapid, and showed 100% typeability (Chuma et al., 1997; Jana et al., 2003). Another advantage of RAPD is that DNA purification is not necessary (Mazurier et al., 1992). However, RAPD-DNA patterns change with template DNA concentration (Gibson et al., 1994). Poor reproducibility (the inherent limitation of all gel-based methods) outweighs the simplicity, rapidness, and cost effectiveness of this method (Fayos et al., 1993; Meunier and Grimont, 1993). Some strains become non-typable due to the variation in DNA extraction and presence of DNase (Fayos et al., 1993). Due to these disadvantages, RAPD is not routinely used in laboratories.

Multilocus-Based Typing Methods

In multilocus-based genotyping methods, multiple loci dispersed throughout the genome come under investigation for genetic polymorphisms. These methods do not reveal the genetic polymorphisms from the whole genome, but multiple markers (ideally distributed around the genome) well represent polymorphisms in the genome and give high discriminatory power.

Multilocus sequence typing (MLST)

MLST is based on the sequencing of a set of essential or housekeeping genes that are present in all strains of a bacterium. This method was first described by Maiden et al. (1998) for Neisseria meningitis based on sequencing of seven slowly evolving housekeeping genes. Since then, the method has been applied to many pathogenic and environmental bacteria (Hannis et al., 2008). When using MLST, the housekeeping genes are amplified by PCR and then the sequence is determined in an automated DNA analyzer. For each housekeeping gene, the different sequence present within a bacterial species is assigned as a distinct allele, and for each isolate, the alleles at each of the loci define sequence type (ST). Allele numbers for new alleles and sequence numbers for new ST are available upon submission of the sequence to the MLST database (http://mlst.zoo.ox.ac.uk/).

The MLST method for C. jejuni was developed by Dingle et al. (2001) based on the sequence of seven housekeeping gene loci—asp, gln, glt, gly, pgm, tkt, and unc—which code for aspartase A, glutamine synthetase, citrate synthase, serine hydroxymethyl transferase, phosphoglucomase, transketolase, and ATP synthase α unit, respectively. MLST is more suitable for long-term and global epidemiological study, as it investigates the variation in slowly evolving housekeeping genes (Dingle et al., 2001; Mickan et al., 2007). It was successfully used in an outbreak investigation of C. jejuni in combination with other typing methods based on antigenic genes such as flaA short variable region (SVR) typing or porA genes (Dingle et al., 2005; Sails et al., 2003b). The initial MLST method for C. jejuni was later supplemented with sequence typing of three more antigenic genes—flaA, flab, and porA—to make it suitable for outbreak investigations (Dingle et al., 2008). This supplementation increased the discriminatory power and made the method suitable for both long- and short-term epidemiological studies (Dingle et al., 2008). However, amplification and sequencing of nine genes makes the process more labor-intensive, time consuming, and costly.

The major advantage of MLST over other genotyping methods such as PFGE is its very high reproducibility due to minimal variation in DNA sequencing (Enright and Spratt, 1998). Global accessibility of data from the continuously expanding database allow electronic portability and interlaboratory comparison of data without the requirement of reference isolates, unlike PFGE. As the MLST can be applied directly to clinical material or extracted DNA, the use of live cultures can also be eliminated (Enright and Spratt, 1998; Maiden et al., 1998). However, some disadvantages of MLST make routine use impractical in public health laboratory. These include the analysis time, cost, and the requirement of pure DNA (Hannis et al., 2008). The risk of contamination of samples from post-PCR manipulation of amplicons is also a disadvantage of MLST. However, semi-automation has made
the method suitable for use in the public health laboratory (Clarke et al., 2001; Sails et al., 2003b).

MLST is a suitable typing method for monitoring of global trends in *C. jejuni* populations, and has been used in a variety of epidemiological studies since its development. Some such studies include diversity and population structure of *C. jejuni* isolates associated with sporadic cases of gastroenteritis (Colles et al., 2003; Mickan et al., 2007; Wilson et al., 2009b), spatiotemporal homogeneity of *C. jejuni* isolates (Rotariu et al., 2009), comparison of veterinary and human isolates (Manning et al., 2001), sources of campylobacter in broiler flocks during rearing (Bull et al., 2006), and source attribution (De Haan et al., 2010; Sproston et al., 2010).

**Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS)–Based Genotyping of *C. jejuni***

Initially, MALDI-TOF-MS, invented in the 1980s by Tanaka in Japan and by Karas and Hillenkamp in Germany independently (Tost and Gut 2006), was applied to protein and peptide analysis. Now, this method is one of the most powerful alternatives to conventional Sanger or pyrosequencing and is a promising genotyping method (Vogel et al., 2009). This method determines sequence variation by comparing the mass of target markers. Initially used to study single nucleotide polymorphism in the human genome (Tost and Gut, 2006), MALDI-TOF-MS was applied for identification and genotyping of bacterial species such as *Acinetobacter baumannii* (Ecker et al., 2006), *Streptococcus pneumoniae* (Williamson et al., 2008), *Helicobacter pylori* (Ilina et al., 2010), and *C. jejuni*. Hannis et al. (2008) described MALDI-TOF-MS–based genotyping of *C. jejuni* using the housekeeping genes of the conventional MLST method as target markers. A short region (<140 bp) of each housekeeping gene is amplified, and base composition of the amplicons is compared by weighing the mass of the amplicons using high-performance mass spectrometry. This method was found to be rapid, high-throughput, and comparably discriminatory with MLST (Hannis et al., 2008). Highly skilled personnel and well-equipped laboratories are required for this method. This newly developed method has yet to be evaluated for epidemiological study.

**Single Nucleotide Polymorphism (SNP) Profiling**

SNPs, the most commonly identified polymorphisms, have often been used as genetic markers for genetic analysis, including genotyping of bacteria (Best et al., 2004, 2005). A genotyping method based on SNP has been developed as a rapid and cost-effective alternative to cumbersome sequence-based methods such as MLST (Hommais et al., 2005; Robertson et al., 2004). The vast amount of comparative sequence data generated from MLST is used to identify SNPs as targets (Rudi et al., 2006). MALDI-TOF-MS, DNA microarray, ligation, electrophoresis, probe hydrolysis, high-resolution melt analysis, and primer extensions are the methods used to identify SNPs (Merchant-Patel et al., 2008). SNP profiling was first used for identification of *C. jejuni* complex 21 by Best et al. (2004). They later used a PCR platform for characterizing a clonal cluster of *C. jejuni* and *C. coli* by SNP profiling. The new scheme was rapid, cost effective, and useful for high-throughput applications (Robertson et al., 2004). This novel strategy for the use of real-time PCR (RT-PCR) for detection and characterization of *C. jejuni* beyond species level supplied real-time epidemiological data comparable with MLST results. Resolution optimized SNP was used separately and in combination with binary gene markers to characterize *C. jejuni* by Merchant-Patel and his group in chickens (Price et al., 2006). The single step method allowed a rapid test of epidemiological linkages in *C. jejuni*. In another seven-member SNP genotyping assay, the method efficiently characterized *C. jejuni* isolates in combination with fla SVR sequencing (Hannis et al., 2008). However, SNP methods had limited discriminatory power unlike full MLST characterization and cannot be used for studies of *C. jejuni* population structure (Best et al., 2004).

**Single Locus–Based Methods**

In such genotyping methods, genetic polymorphism is investigated in only one locus, either by sequencing of the locus or by restriction profiling. These methods are least representative of the polymorphisms in the strains. The fla gene is the most studied locus for single locus genotypic methods for *C. jejuni* in which recombination has been shown to occur (Petersen and Newell, 2001). Other loci studied include the LOS gene cluster (Shi et al., 2002). *Campylobacter* spp. are naturally competent to take up and incorporate DNA, and so any genotyping technique based on a single genetic locus may provide inaccurate results for *C. jejuni* (Duim et al., 1999). For this reason, single locus–based methods are mostly used in preliminary screening of large numbers of strains in epidemiological studies.

**flaA–restriction fragment length polymorphism (flaA-RFLP)**

flaA–RFLP typing is one of the simplest and most cost-effective genotyping methods for the investigation of large number of *Campylobacter* spp. (Petersen and Newell, 2001). Widely used due to its rapidity and simplicity, the technique involves PCR amplification of the flagellin gene locus and restriction enzyme digestion to generate simple restriction fragment length polymorphic fingerprints. The flagellin locus has two genes, *flaA* and *flaB*, arranged in tandem (Nuijten et al., 1990), and the presence of both highly conserved and variable regions in the flagellin locus of *C. jejuni* makes it a suitable genetic marker for RFLP of a PCR product (Meinersmann et al., 1997). A number of *fla* typing procedures have been developed and reviewed previously (Wassenaar and Newell, 2000). These methods vary in the manner of DNA preparation, primer design, annealing temperature, restriction enzyme used, and nomenclature (Wassenaar and Newell, 2000). Most of the methods developed use only *flaA* (Nachamkin et al., 1993; Nachamkin et al., 1996; Santesteban et al., 1996), and both *flaA* and *flaB* were used by Ayling et al. (1996) and Harrington et al. (2003). Optimal discriminatory power was obtained when both genes were amplified separately (Petersen and Newell, 2001). This method can be used in combination with other genotyping methods such as MLST when the strains need further subtyping (Djordjevic et al., 2007).

**fla short variable region (SVR) sequence typing**

This *C. jejuni* genotyping method based on the *flaA* SVR was developed by sequencing 267 bp SVR of the *flaA* gene
This is a simple and useful variation of flaA-RFLP method with high sample throughput at reasonable cost (Meinersmann et al., 2005). As flaA-SVR sequence typing is a single gene–based method, it is not suitable for long-term, time/location-based epidemiological studies. However, the presence of two copies of fla gene and possible intragenomic and intergenomic recombination between the two copies undermines the reliability of typing Campylobacter spp. (Cornellius et al., 2010; Guerry et al., 1991). Despite this, it is recommended for preliminary screening of large number of C. jejuni isolates. It was found useful in combination with other typing methods such as MLST to differentiate closely related or outbreak isolates (Dingle et al., 2005; Sails et al., 2003b).

**High-resolution melting (HRM) analysis of flaA fragment**

Another flaA gene–based genotyping method for C. jejuni evaluated very recently by Merchant-Patel and group, this is a method based on real-time PCR (RT-PCR). Here, reduction in fluorescence of PCR product stained with double-strand–specific dye was monitored while the product was heated through its melting temperature (Merchant-Patel et al., 2010). flaA HRM can be used for onsite screening of a large number of C. jejuni isolates.

**Repetitive Sequence PCR (rep-PCR) Analysis**

Rep-PCR is a bacterial genome fingerprinting method first described by Versalovic et al. (1991) based on PCR amplification and gel electrophoresis of repetitive DNA elements present within bacterial genome. Two main sets of repetitive elements used in such subtyping are 38-bp repetitive extragenic palindromic (REP) and 128-bp enterobacterial repetitive intergenic consensus sequences (ERIC). Another repetitive DNA element, BOX element, is also used for bacterial genotyping. Extracted DNA or whole bacterial cells can be used in this typing; methods that use whole cells have the advantage of being quicker (Woods et al., 1993). A single primer, a single set of primers, or multiple sets of primer can be used for amplification of REP and ERIC elements. Typing can be done by amplifying either REP or ERIC element, but combining both elements results in more discriminatory power (Olive and Bean, 1999). Moser et al. (2002) applied the ERIC-PCR method to investigate the diversity of C. jejuni in human, poultry, and other sources along with PFGE and Penner typing. The method represented genetic relatedness of the strains in relation to their host origin better than PFGE with Smal (Moser et al., 2002). When the discriminatory power of these three genotyping methods, PFGE, MLST, and rep-PCR, were assessed with 63 C. jejuni isolates recovered from chickens raised in conventional, organic, and free-range poultry flocks, combined amplification of ERIC, REP, and BOX elements (repREB-PCR) showed greater discriminatory power than PFGE and MLST (Wilson et al., 2009b). On the other hand, ERIC-PCR and BOX-PCR produced the highest number of PCR products and greatest reproducibility (Wilson et al., 2009a). Further evaluation of this method is required.

**PCR–denaturing gradient gel electrophoresis (PCR-DGGE)**

PCR-DGGE analysis distinguishes bacterial isolates based on the sequence differences in a single gene. Hein et al. (2003) applied this chemical denaturing gradient method to genotype C. jejuni using the fla gene sequence as genotyping marker. In this method, the variable fragment of 170–210 bp from the 3’ end of flaA to the 3’ end of the intergenic region that separates the tandemly oriented flaA and flaB gene of C. jejuni was the target marker. A commercially available denaturing gel electrophoresis system was used in this method, which was suitable to detect nearly all single-base as well as multiple single-base substitutions in DNA fragments (Farnleitner et al., 2000; Myers et al., 1985; Sheffield et al., 1989).

This is a rapid method suitable for primary subtyping of C. jejuni (Hein et al., 2003). Najdenski et al. (2008) considered this cultivation-independent subtyping method suitable for primary screening of C. jejuni and C. coli when studied in community cecal samples from broilers.

**PCR–single strand conformation polymorphism (PCR-SSCP)**

SSCP is a culture-independent single gene–based rapid genotyping method. This method was first developed for detection of polymorphisms in human DNA. In SSCP analysis, single-stranded DNA fragments are differentiated according to differences in their electrophoretic mobility due to sequence-dependent conformational changes of the fragments (Orita et al., 1989). Charvalos et al. (1996) applied this method for detecting gyrA mutations associated with ciprofloxacin resistance in C. jejuni. This method later was used to investigate C. jejuni infection in an immunocompromised patient in a comparative study using Penner serology, Preston biotyping, phage-typing, RFLP, 16S ribotyping, and flaA RFLP. PCR-SSCP was found to be useful in terms of rapidity, discriminatory power, and simplicity in C. jejuni outbreak characterization but not suitable for examination of the clonal patterns over a long period of time (Moore et al., 2001). PCR-SSCP, as a rapid subtyping method, was validated by subtyping of 48 clinical C. jejuni isolates, 49 C. jejuni strains from poultry, two strains from ducks, and one strain from a pheasant. This method can also be applied to C. coli, as C. jejuni and C. coli share flagellin gene types (Hein et al., 2003).

**Ribotyping**

Ribotyping has become a well-validated and reproducible means of distinguishing between strains in many bacterial genera such as Pseudomonas, Salmonella, Haemophilus, Mycobacterium, Legionella, and Campylobacter (Owen, 1989). Foyas et al. (1992) found the method effective in identifying strains of C. jejuni with 100% typeability. This technique involves electrophoresis of digested whole genome followed by Southern blot hybridization with probes specific for rRNA. EcoRI is the most frequently used restriction enzyme for digestion (Pavlic and Griffiths, 2009). Other enzymes such as PstI, HaeIII, HinFI, and PvuIII are used alone, or in pairs or in combination (Wassenaar and Newell, 2000). Manual ribotyping is labor intensive and time consuming, and an experienced technician is required to maintain consistent protocol for meaningful results. All these drawbacks make this method
unsuitable for routine surveillance (Pavlic and Griffiths, 2009). To overcome these limitations, automated ribotyping (AR) systems have been developed. The RiboPrinter® Microbial Characterization System™ was commercialized by Qualicon (Wilmington, DE) as an AR instrument capable of performing 32 assays per day. In the RiboPrinter® system, the instrument conducts cell lysis, nucleic acid digestion, Southern blotting, and collection of digitized hybridized DNA patterns (Arvik et al., 2005). This automation has reduced the required time and labor, but AR is not a suitable method for routine surveillance for C. jejuni, as it has shown 98.7% typeability (Fussing et al., 2007) and is much less discriminatory (only 50%) than PFGE (Ge et al., 2006). Ribotyping was not recommended as a method of choice for characterization of C. jejuni (Pavlic and Griffiths, 2009), but it can be used in combination with other methods such as MLST or RFLP of the flaA gene (O’Reilly et al., 2006). Ribotyping was used in epidemiological study alone or in combination with other methods such as in studying distribution of predominant C. jejuni strain in slaughterhouse environment (Klein et al., 2007), genotyping diversity in wild bird community (Waldenstram et al., 2007), and source attribution of sporadic infection (Nielsen et al., 2006).

Future Directions

Molecular typing systems are evaluated based on their performance (efficacy) and convenience (efficiency) criteria (Struelens, 1996). Major performance criteria are typeability, reproducibility, stability of markers and discriminatory power, and epidemiological concordance, whereas convenience criteria are flexibility, rapidity, accessibility, and ease of use.

No method possesses all these criteria at a satisfactory level, and each method has advantages and disadvantages. Thus, no single typing method has yet been found to be universally applicable for effective epidemiological application (Olsen et al., 2001). At least two genotyping methods should be combined to accurately address questions of bacterial lineage and epidemiological issues (Wassenaar et al., 1998). The search for new subtyping methods continues, and there is room for the development of a new sequence-based method for C. jejuni. As none of the presently available genotyping methods for C. jejuni investigates virulence and virulence-associated genes for epidemiological studies, such a method could be developed for both short- and long-term epidemiological studies (Chen et al., 2007; Zhang et al., 2004).

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