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Multi-locus sequence typing of *Campylobacter jejuni* using a novel polymerase-chain-reaction-microsphere method

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Introduction: Campylobacter is a major cause of foodborne disease, with *Campylobacter jejuni* contributing more than 90% of reported cases. For diagnosis and monitoring of transmission, several genotyping methods have been developed, including multi-locus sequence typing (MLST). However, there is still a need for fast, cost effective methods for routine analysis. We developed a technique combining allele-specific PCR with a microsphere-flow cytometer system. Seven loci with single-nucleotide polymorphisms (SNPs) with the highest Simpson's index of diversity (D) were selected from an MLST database. With these loci as a target, multiplex allele-specific PCR was conducted on microspheres in a single reaction and fluorescence signal was detected in a flow cytometer. The signal on the microspheres indicated which allele specific primer was consumed in the PCR. By this means all seven loci could be determined. This approach has a turnaround time of 4 h.

Results: To date, the method has been tested with two strains of *Campylobacter jejuni* possessing known SNP patterns and six of seven loci have, at time of writing, been correctly determined for these strains, in a single PCR reaction.

Conclusion: A new allele specific PCR-microsphere-based method for genotyping *Campylobacter jejuni* is under development. This approach will be useful in laboratories utilizing PCR and flow cytometers.

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