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Campylobacter fact sheet: taxonomy, pathogenesis, isolation, detection and future perspectives

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Abstract

Background Campylobacter is the most common bacterial cause of gastro-intestinal disease worldwide with consumption of contaminated food or water typically being cited as principal sources of infection. *Aims* To inform the efforts of the EC-funded MoniQA project team investigating the harmonization and standardization of analytical methods for foods, we prepared a brief review of key aspects of these bacteria, encompassing taxonomy, behaviour in foods, clinical symptoms, isolation, detection, identification and subtyping. A summary of future needs and perspectives to help protect public health from this important human food- and water-borne pathogen is also given.

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Introduction

Campylobacter has been identified as the leading cause of bacterial zoonotic enteric infections in developed and developing countries worldwide (WHO, 2000). Many risk factors for *Campylobacter* have been identified. In developing countries, inadequately treated water and contact with farm animals are assumed to be important in outbreak situations. In developed countries, the pattern of transmission appears to be more complex. The consumption of contaminated, undercooked poultry in particular is cited as a leading cause of food-borne infection. Other meats may also be potential sources of food-borne infection and, although relatively uncommon, contaminated water and milk are the most common causes of common-source outbreaks (http://www.who.int/topics/campylobacter/en/).

There are presently 22 species and eight defined subspecies assigned to the genus *Campylobacter* (On, 2005; Inglis *et al*., 2007; Debruyne *et al*., 2009, 2010; Rossi *et al*., 2009; Zanoni *et al*., 2009), of which the most frequently reported in human disease are *Campylobacter jejuni* (subspecies *jejuni*) and *Campylobacter coli*. *Campylobacter lari* and *Campylobacter upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease (http://www.who.int/mediacentre/factsheets/fs255/en/print.html). These species are often collectively referred to as the 'thermotolerant campylobacters' due to their ability to grow at 42 °C. The majority of *Campylobacter* species are microaerophilic, motile, curved S – or spiral-shaped Gram-negative rods, 0.2–0.8 µm wide and 0.5–5 µm in length. The optimum growth temperature of thermotolerant *Campylobacter* lies between 35 and 42 °C.

Campylobacter is carried in the intestinal tract of a variety of wild and domestic animals, and as a result of faecal

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Table 1 Comparison of reported human campylobacteriosis rates between selected countries

	Campylobacter rate/ 100 000 population		
Country	2006	2007	References
Australia[1]	112.4	120.2	OzFoodNet (2007, 2008)
Europe (including non-EU member states)	46.1	45.2	EFSA (2007, 2009)
New Zealand	383.5	302.2	ESR (2007, 2008)
USA[2]	12.68	12.78	CDC (2006, 2007)

[1]Excludes New South Wales which only reports campylobacteriosis when an outbreak occurs.
[2]Data collected from 10 US States (Foodnet).

contamination during production or processing, may contaminate foods derived from these animals. Refrigeration may promote survival, while freezing, although causing a reduction in numbers, does not eliminate the bacterium. Cooking readily destroys the organism, and it is particularly susceptible to drying (Wallace, 2003). Growth does not occur below 30 °C, so actual multiplication during handling or storage at room temperature will not occur in moderate climates (Jacobs-Reitsma, 2000).

The most common symptoms of *Campylobacter* infection include diarrhoea, abdominal pain, fever, headache, nausea and vomiting. Symptoms typically start 2–5 days after infection and last for 3–6 days. Hospitalization due to *Campylobacter* infection has been estimated at approximately 8–10% of notified cases, or 0.5–0.6% of all community cases. Mortality from campylobacteriosis has been estimated at a case-fatality ratio of 1 per 10 000 (data reviewed in a study estimating the burden of campylobacteriosis in the Netherlands: Havelaar *et al*., 2000). Specific treatment is not usually necessary, except to replace electrolytes and water lost through diarrhoea, but antimicrobials (including erythromycin, tetracycline, quinolones) may be needed to treat invasive cases and the carrier state. Severe complications, such as Guillain–Barre syndrome (GBS) and Reiter's syndrome reactive arthritis may be chronic sequelae to *Campylobacter* infection. Table 1 shows a comparison of reported campylobacteriosis rates between selected countries worldwide.

Survey of methods

A wide range of methods currently exist for the detection, culture, identification and differentiation of thermotolerant *Campylobacter* to the genus, species and strain level. Two comprehensive reviews have recently summarised methodology relating to isolation procedures (Donnison, 2003) and phenotypic and genetic typing methods (Klena, 2001). A summary of some of the phenotypic and genetic methods, together with their advantages and disadvantages, is listed in Table 2. Most methodological developments are directed at the thermophilic species because these are more frequently detected in human disease. One EC-funded project (Keevil, 2006) did, however, produce some advances in developing broader methods suited to recovering a wider range of species, with the premise that several non-thermophilic species could also be important pathogens.

While there appears to be no 'standard' method for isolating campylobacters from food, faeces or environmental samples, protocols have been published by several recognized authorities including the International Standards Organization (ISO, 1995), the UK Public Health Laboratory Services and the US Food and Drug Administration (FDA, 1998). A report to the UK Microbiological Safety of Food Funders Group (MSFFG, 2004) has recently provided an overview of progress in publicly funded *Campylobacter* research in the United Kingdom. In addition to the principal areas of research in genomic classification of strains, genetics and physiology, pathogenicity, epidemiology and risk analysis, the report also summarises current gaps in research and knowledge.

There are several widely used methods for isolating *Campylobacter*. For food and environmental samples, which are usually contaminated with relatively low bacterial numbers, the incorporation of pre-enrichment procedures using broth culture, for example Preston broth, Exeter broth, Bolton broth, *Campylobacter* enrichment broth or Park and Sanders broth, have been found to increase recovery of *Campylobacter* from most sample types. Procedures may include the use of a resuscitation period at a reduced temperature before increasing the temperature for the remainder of the incubation time, and the inclusion of oxygen-quenching supplements in pre-enrichment media. For analysis of faecal samples from infected patients, direct plating onto either blood agar or a *Campylobacter* selective agar is often preferred to pre-enrichment in view of the higher numbers of strains present in clinical samples. The review by Donnison (2003) summarises the advantages and disadvantages of various broths and agars currently used for *Campylobacter* isolation.

Phenotypic and genetic methodologies are becoming increasingly popular for the direct detection and identification of *Campylobacter* species in samples. These methods often include a conventional step to increase the recovery of

injured cells and to allow isolates to be obtained in pure culture for subsequent typing. Of these, PCR predominates in research and reference laboratories while the more cost-effective ELISA methods are typically used in routine laboratories (http://www.laborlifescience.com.br/artigos/campylobacter.pdf; Sails *et al*., 2001). Most PCR assays are directed at detecting one or a few species, although a PCR-based direct detection approach using denaturing gradient gel electrophoresis has been successfully used to detect and identify an extensive taxonomic range of campylobacters and related organisms, including *Helicobacter* and *Arcobacter*. These have been identified from complex ecosystems including saliva and the gastro-intestinal tract in a variety of host species including humans, dogs, cats and different species of zoo animals (Abu Al-Soud *et al*., 2003; Waleed *et al*., 2006; Petersen *et al*., 2007). Immunomagnetic separation (where magnetic beads coated with *Campylobacter*-specific antibody are used to specifically recover strains in complex matrices) can also be used to help concentrate cells to recoverable or detectable quantities. In addition, nested- or semi-nested PCR approaches can enhance the sensitivity of assays, especially when combined with more robust polymerases.

The taxonomy of *Campylobacter* and related organisms (e.g. *Arcobacter*, *Helicobacter*) is complex and as a result, identification to species level is not straightforward (reviewed by On, 2005). Standardization of phenotypic/biochemical tests used for this purpose is highly recommended, and the accuracy of genetic approaches such as PCR or sequencing requires careful consideration of the organism's taxonomy before methods or results can be deemed valid.

Epidemiological subtyping of strains has seen substantive developments. The phenotypic methods (serotyping, phage typing, biotyping) first developed in the 1980s were found to be inadequately discriminatory and insufficiently portable. Consequently, a plethora of genetic methods have been described, with varying degrees of success (Table 2). Of methods currently available, Pulsed-field gel electrophoretyping (PFGE) (Figure 1) and Multi Locus Sequence Typing (MLST) have become increasingly used because methods are well standardized and databases for international comparison available. They are not, however, inexpensive and their routine application is far from trivial.

The PFGE method involves the digestion of whole-cell DNA with so-called 'rare cutting' restriction enzymes, generating relatively few genomic fragments of a comparatively large size. The DNA fragments are separated by a special electrophoretic method, which involves the co-ordinated application of pulsed electric fields from three different directions, one through the central axis of the gel, and two that run at an angle of 120 degrees either side. The pulse times are equal for each direction resulting in a net forward migration of the DNA with the result that better resolution of large DNA molecules is achieved.

MLST directly measures the DNA sequence variations in a set of housekeeping genes and characterizes strains by their unique allelic profiles. The technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at different numbers of genes depending on the level of discrimination required.

Future perspectives and conclusions

Currently, laboratory methods used for the isolation and detection of *Campylobacter* are generally suitable for thermophilic species found in clinical samples. However, problems still remain when isolating *Campylobacter* from food and environmental samples where bacterial numbers may be particularly low. In addition, methodological bias often obstructs the recovery of certain species, notably *C. upsaliensis*. Consequently, there is often little standardization or harmonization of methodology globally. Published rates per 100 000 population assigned to different countries should be interpreted with appropriate caution.

The identification of different species can be complex and to date, limited progress has been made with the detection of species other than *coli* or *jejuni*, some of which may well prove to be important in human cases associated with food-borne infection. Currently, there also appears to be limited awareness of the relevance of designing new phenotypic and/or genotypic assays. Indeed, recommendations for performing biochemical tests, while widely published, may not always be heeded.

While a multitude of subtyping methods have been described for *C. jejuni*, accurate and sensitive methodology is pivotal for the recognition of outbreaks of infection, for matching case isolates with those from potential vehicles of infection and for discriminating these from unrelated strains. Many of the methods described are of limited value for accurate surveillance and identification of infectious sources due largely to variable discriminatory properties or limited availability. The wider application of genetic methods for typing campylobacters, including PCR-based methodology, has facilitated a higher level of discrimination with the caveat of a lack of standardization. Results from one laboratory cannot easily be compared with those from another, which significantly reduces their usage for

Table 2 Summary of phenotypic and genetic methods used to identify thermotolerant *Campylobacter* spp. strains (Klena, 2001)

Method	Advantages	Disadvantages
Biotyping	Excellent typeability[1] Easy to use Easy to interpret Reagents readily available Low cost	Poor reproducibility[2] Poor discriminatory power[3]
Resistotyping	Excellent typeability Good reproducibility Easy to moderately labour intensive Easy to moderately hard to interpret Reagents readily available Low cost	Poor discriminatory power
Bacteriophage typing	Easy to moderately labour intensive Easy to moderately hard to interpret Reagents generally available Moderate cost	Variable typeability Fair reproducibility Variable discriminatory power
Protein profiling	Excellent typeability Good reproducibility Excellent discriminatory power Reagents generally available Moderate cost	Skilled technician necessary Interpretation easier with computer-assisted software
Fatty acid methyl ester profiling	Excellent typeability Good reproducibility Very good discriminatory power	Requires extensive equipment Skilled technician necessary High processing time
Serotyping	Good reproducibility Moderate costs	Variable typeability Variable discriminatory power Moderate/high costs to maintain Variable interpretations
Plasmid profiling	Low to moderate cost Reagents available universally	Variable typeability Fair reproducibility Variable discriminatory power Skilled technician necessary Result interpretation often difficult
Restriction endonuclease analysis	Very good/excellent typeability Reagents available universally Low to moderate costs	Variable reproducibility Variable discriminatory power Skilled technician necessary Result interpretation often difficult Requires computer software
Hybridization methods (e.g. ribotyping)	Excellent typeability Excellent reproducibility Good discriminatory power Moderate costs Reagents available universally	Skilled technician necessary Moderate/high cost per sample Low throughput
Macrorestriction methods (e.g. PFGE) (Figure 1)	Excellent typeability Excellent reproducibility Excellent discriminatory power Moderate costs Reagents available universally	Initial equipment costs expensive Computer software advantageous Low sample throughput Skilled technician necessary
Polymerase chain reaction methods	Excellent typeability Good to excellent reproducibility Good/excellent discriminatory power Moderate costs Reagents available universally	Variability in methodology Skilled technician necessary Dedicated facilities necessary to avoid cross-contamination
Multilocus sequence typing	Excellent typeability Good to excellent reproducibility Good/excellent discriminatory power	Expensive Skilled technician necessary Requires computer software Low throughput Time consuming

[1]Typeability refers to the ability of the system to give an unambiguous positive result for each isolate.

[2] Reproducibility refers to both the method's ability to give the same result when one tests the same isolate repeatedly and also to the typed attribute's stability over time.

[3]Discriminatory power refers to the test's ability to differentiate epidemiologically unrelated strains.

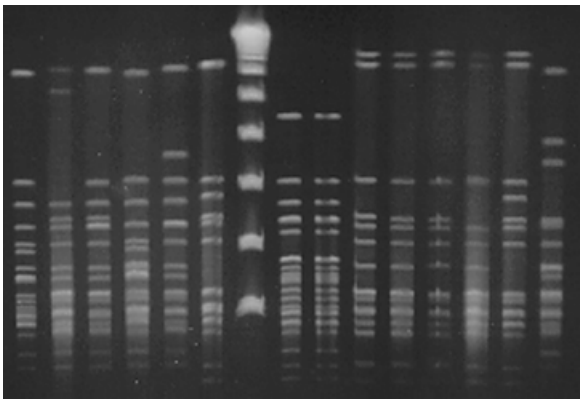

Figure 1 *Kpn*I-based pulse-field gel electrophoresis – DNA profiling of Danish *Clostridium jejuni* strains (http://campynet.vetinst.dk/).

investigating the complex issue of *Campylobacter* epidemiology at national and international levels. In New Zealand, a microbial typing database has been established which aims to harmonize PFGE methodology for *Campylobacter* on a nationwide basis, but to also ensure that it is internationally comparable. The database, which is compatible with the PulseNet USA system (http://www.cdc.gov/pulsenet) allows submission of serotyping, PFGE and epidemiological data on *Campylobacter* isolates to a centralized server. Additionally, the *Campylobacter* MLST website contains linked databases for both allelic profiles and sequences and isolate information http://pubmlst.org/campylobacter/. The major drawback with implementation of PFGE and MLST remains cost. For example, inter-laboratory comparisons require strict adherence to complex, standardized protocols, thereby limiting such comparisons with laboratories that have access to the appropriate equipment and software. The Institute of Environmental Science and Research Ltd. has developed an alternative binary typing approach based upon PCR-based detection of 18 specific marker genes mostly associated with epidemicity (Cornelius *et al*., 2010). This 'P-BIT' system has the potential for international comparability, high discrimination but low set up and running costs.

It would seem likely that 'conventional' methods will continue to have a place in the isolation and identification of campylobacters. However, as the genomes of many *Campylobacter* species have now been sequenced, this will help to identify species-specific loci, the products of which may help to develop and validate easy and quick diagnostic tools. Global cooperation, sharing of strains and databases should help to close any existing gaps in *Campylobacter* identification tools. Additional methods may be required if a wider range of non-thermophilic campylobacters are confirmed as important human pathogens.

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