Microbial update

Campylobacter

Produced as a service to the food industry by Oxoid Ltd.

Campylobacter is the most frequently reported bacterial cause of infectious gastroenteritis in the developed world. As such it represents a huge economic burden to society and yet, compared to other foodborne pathogens, testing for its presence/absence is often not routine.

Campylobacter species occur naturally in the gastrointestinal tract of wild birds and animals (both domestic and wild) and because of this, they are ubiquitous within the environment. The transfer of campylobacter to humans has, not surprisingly, been associated with a whole host of sources including poultry, raw meat and water. In addition, although the organism does not readily multiply in foods, it has a very low infective dose (less than 500 cells).

Thankfully, campylobacter are sensitive to the high temperatures associated with cooking and pasteurisation and with good hygiene in the kitchen, can be readily controlled. One interesting outbreak in the UK involved milk delivered to the doorstep early in the morning. The foil seal on the bottles had been pecked open by birds. The birds were carriers of campylobacter and therefore contaminated the milk.

Unwittingly, people were pouring contaminated milk directly onto their breakfasts and into their tea.

With salmonella and listeria testing and sampling regimes well established, the focus is slowly changing to address the issue of campylobacter. For example, in 2000, the Food Standards Agency (FSA) in the UK set a target to reduce the incidence of foodborne illness by 20% by 2006. As the major bacterial cause of foodborne disease, it was considered that a reduction in human campylobacteriosis would contribute significantly to this target.

The organism

Campylobacter comprises three thermotolerant (they can grow at temperatures >40°C) pathogenic species: C. jejuni, C. coli and C. lari.

Other campylobacter species such as C. upsaliensis have occasionally been implicated in human disease but whether this is due to contaminated food is unclear. A host of other thermo-sensitive, non-pathogenic species also exist.

All are Gram negative, oxidase positive, spiral or curve shaped rods. They are motile, with a characteristic twisting movement. The majority of campylobacter species are microaerophilic, requiring reduced oxygen levels of 3-5% and carbon dioxide levels of 2-10% to survive and grow. This, and their sensitivity to environmental stresses make them relatively difficult to isolate.

Campylobacteriosis

Campylobacter infection in humans causes mild to severe diarrhoea, often accompanied by fever, headache, aching muscles and nausea. The disease is usually self-limiting, with most people making a full recovery within 7-10 days.

However, as mentioned, this can create a severe economic burden.

Rarely, campylobacter infection can lead to more serious complications. Such long term sequelae include rheumatological (reactive arthritis), neurological (Guillain-Barré Syndrome) and renal (haemolytic uraemic syndrome) problems. Guillain-Barré syndrome is the most severe complication and occurs about once in every 1000 cases of human campylobacter infection.

This is an acute bilateral paralysis, which may develop 1-3 weeks following the onset of diarrhoea. It may last for several weeks and usually requires intensive care.

Campylobacter can also cause life threatening septicaemia in immuno-compromised individuals.

Campylobacter in poultry

Although campylobacter is found in a number of different foods, there is strong evidence that poultry is the most significant food source for human campylobacter infection. Broiler chickens in particular, can carry large numbers of the organism without illness. C. jejuni and, to a lesser extent, C. coli are common in commercial poultry flocks.

Indeed, up to 60% of housed poultry flocks are positive for campylobacter at slaughter age and an FSA survey in 2001 demonstrated that 50% of raw chicken samples (fresh and frozen) purchased at retail outlets in the UK were contaminated with campylobacter. Similar contamination rates have been anecdotally reported in other European countries (with the exception of Scandinavia).

A reduction in poultry related campylobacter illness could be tackled in two ways: by reducing the number of infective cells on the meat/carcase or by reducing the number of contaminated birds. The former can be achieved to some extent by freezing, heat treatment (hot water washes) or irradiation. However, the latter is preferable in order to reduce the burden of the organism on commercial and domestic kitchens.

In the past, it was thought that the control of campylobacter in poultry flocks was unrealistic due to the ubiquitous nature of the organism and its widespread presence in wild birds and animals. However, recent evidence suggests that the reduction of campylobacter in housed broiler chickens is now a practical proposition.

Intervention measures, such as good farming and biosecurity practices in Scandinavia have been successful in controlling campylobacter in housed birds. In Norway, for example, targeted on-farm intervention has reduced campylobacter in fresh poultry products to <10% (it was as low as 2% in 2002).

Similarly, biosecurity measures in Swedish poultry farms has resulted in a reduction of campylobacter in flocks to <10%.

Continued on page 15

Table 1. Differentiation of campylobacter species.

Characteristic	C. jejuni	C. coli	C. lari
Microaerobic growth at 25°C	-	-	-
Microaerobic growth at 42°C	+	+	+
Aerobic growth at 42°C	-	-	-
Catalase	+	+	+
Hippurate hydrolysis	+	-	-
Motility	yes	yes	Yes
Gram stain	G –ve, curved rods	G –ve, curved rods	G –ve, curved rods
Indoxyl Acetate Hydrolysis	+	+	-
Naladixic acid	Sensitive	Sensitive	Resistant

Continued from page 13

These Scandinavian models have been trailed in the UK with some success and so research and investigation in this area of control are likely to continue.

Throughout the food chain

The World Health Organisation states that the prevention of campylobacter infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in commercial and domestic environments.

For raw products, the risk of campylobacter infection can be eliminated by the consumer cooking the product. Whilst producers and manufacturers must take responsibility for reducing the number of contaminated products reaching the supermarket shelf, the end user must also ensure they follow hygienic food preparation practices and carry out effective cooking in the kitchen to ensure they do not contract campylobacteriosis.

For ready-to-eat products however, the end user will consume the product without any intervention. Thus, it is extremely important for the manufacturer to ensure that the product is free from campylobacter.

In these situations the source of campylobacter contamination is likely to be environmental or cross-contamination, and so good hygiene practices, including HACCP control measures, throughout food production and preparation should eliminate any risk. Guidelines for those involved in food preparation are widely available.

Methods of detection

Enrichment

Campylobacter is relatively difficult to grow in the laboratory because of its fragile nature and its requirement for microaerobic conditions. In the examination of food products, processing and exposure to the environment can cause sub-lethal injury to campylobacter species.

It is therefore necessary to perform an enrichment step (for 44 hours in Bolton Broth, according to ISO 102727) to allow the resuscitation, recovery and multiplication of injured (and healthy) organisms.

Most enrichment broths also contain a cocktail of antibiotics and other selective agents. Without these, other bacteria would proliferate and mask the presence of any campylobacter in the original sample.

Isolation and identification

Once enriched, a number of selective plating media are available for the isolation of campylobacter species. These include modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA), Karmali Agar, Skirrow Agar or Preston Agar.

Inoculated plates require micro-aerobic incubation for 44 hours (a total of four days including enrichment) according to ISO 10272, after which suspect colonies may be further examined in order to identify the campylobacter species. Identification can be performed by biochemical, morphological or physiological means.

The main pathogenic species of campylobacter can be identified by the criteria shown in Table 1. Some of these tests can be difficult to carry out and interpret; some are also quite laborious and time consuming. Several rapid methods, therefore, have become available to give a rapid and accurate, presumptive identification.

The Dryspot campylobacter test from Oxoid is a latex agglutination test utilising sensitised particles. These particles are coated with specific antibodies and visibly agglutinate in the presence of campylobacter. Oxoid has recently launched an alternative test for the presumptive identification of campylobacter. The OBIS campy test combines a Gram lysis test with detection of the enzyme L-alanyl aminopeptidase (L-ALA).

Campylobacter species are Gram negative and L-ALA negative. Both tests take less than two minutes and are easy to carry out and interpret.

Some laboratories, however, require a complete identification of the organism, which can only be achieved by a full biochemical profile. Whilst this is a long and tedious process, thankfully it is not routinely required. Nevertheless, some commercial kits are available to make this process easier such as API from bioMérieux.

Micro-aerobic conditions

Large cabinets with a modified environment and pressurised gas cylinders are sometimes used to achieve the micro-aerobic conditions necessary for the culture of campylobacter.

More often, however, specific gas generating devices such as the Oxoid CampyGen can be activated in airtight chambers containing broth/plates to produce the necessary atmosphere.

Rapid detection methods

So far, the cultural detection of campylobacter –which can take four days or more – has been discussed. It is well recognised that immunological and molecular methods allow the detection of the micro-organism more quickly than traditional culture methods.

However, immunological methods require the expression of target antigens and thus can be prone to false-negative results.

Conversely, other organisms can carry similar antigens and give rise to false-positives. By detecting the coding DNA of the organism, generic methods such as polymerase chain reaction (PCR) largely overcome this issue. Until recently, such methods were not available to routine testing laboratories.

However, simple methodology and automation is now allowing food laboratories to consider methods such as PCR to detect a wide range of foodborne pathogens. PCR tests for food pathogens including campylobacter are available form several manufacturers.

One of the easiest and most user friendly PCR methods currently available is the BAX System Q7 from DuPont Qualicon. It does not require any centrifugation steps and all the reagents are supplied ready to use in a single tablet.

Further investigation

Currently, there are no clear criteria for acceptable levels of campylobacter in foods. This is largely due to historical difficulties in controlling the pathogen; the fact that it is endemic in many foods and the environment and that it is easily killed by cooking.

However, the success of 'on-farm' control measures, combined with increasing attention by health authorities means the need for food microbiology laboratories to test for campylobacter is likely to increase.

Research into campylobacter is increasing in order to find ways of reducing the incidence of contamination and illness. The ability to easily quantify and differentiate the different campylobacter species is important in this task: for assessing levels of contamination; in determining the source of a contamination problem and in monitoring the effectiveness of the campylobacter management process.

New PCR methods that allow quantification and differentiation will help in this task.

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