=Special Lecture=

Campylobacter Infections: Food-borne Sources and Isolation Methods

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Introduction

The Campylobacteriaceae comprises *Arco-bacter* spp., *Campylobacter* spp. and *Helicobacter* spp. *Campylobacter* species are microaerophilic, gram negative spiral shaped organisms and are found in various anatomical sites of many animals, birds and man but most of these do not cause disease in man. However, the thermolerant/thermophilic campylobacters are a major cause of bacterial gastroenteritis and food poisoning. (Wheeler *et al.*, 1999; Mead *et al.*, 1999) Human clinical infection is most frequently caused by *Campylobacter jejuni* and *C. coli*. These two species are carried in the gastro intestinal tract of many animals and birds including poultry and wild birds and hence are major zoonotic pathogens. (Humphrey *et al.*, 2007). Infection with *C. lari* and the related urease positive thermophilic campylobacters (Bolton *et al.*, 1985). These campylobacters are particularly associated with the marine environment and shellfish (Matsuda and Moore, 2004).

Infection with *C. jejuni/coli* is the most common cause of bacterial gastroenteritis and is characterised by an incubation period of 2–5 days following ingestion of the organisms, resulting in profuse watery diarrhoea which is accompanied by fever, severe abdominal pain and about 50% of cases present with bloody stools. These gastrointestinal symptoms may last for 3–7 days and in rare cases excretion may last for several weeks. Whilst the predominant disease is gastro-intestinal other infections have also been reported but less commonly. These include: bacteraemia, which occurs approximately 1 in every 500 cases, meningitis, Haemolytic Uraemic Syndrome, cholecystitis, pancreatitis, hepatitis, peritonitis, myocarditis,

abscesses. Some cases develop post infection non-enteric sequelae about three weeks after the initial infection. The most important of these are Guillain Barre Syndrome (GBS) an acute demyelinating peripheral neuropathy, Miller Fisher Syndrome and reactive arthritis (Nachamkin, 2002). GBS is now the most common cause of flaccid paralysis worldwide and occurs in about 1 in 1,000 infections and is associated with Heat Stable (Penner) serotypes HS19 and HS41 (Nachamkin, 2002) and results in hospitalisation of affected patients. GBS is well documented as a problem in Japan (Takahashi *et al.*, 2005). The infectious dose for *Campylobacter* infection is known to be relatively small and about 500 organisms have been reported to initiate infection. (Anonymous, 2005) This may be one of the main reasons why infection with *Campylobacter* is so common.

There are no global systems for studying the human epidemiology of *C. jejuni* infection and therefore the best epidemiological information is recorded from countries with integrated clinical and public health surveillance systems. The burden of disease in the USA has been estimated at 2.4 million cases/year with 13,000 hospitalised (Mead *et al.*, 1999) and in the UK as 500,000 case per year (Wheeler *et al.*, 1999). The majority of these infections are believed to be attributed to food-borne transmission. In most developed countries there is a seasonality associated with infection. There is a steep rise in the number of cases in spring whilst the peak is reached in early summer (Adak *et al.*, 1995). Although there are many isolated or sporadic cases of infection outbreaks of infection are relatively uncommon but may be under reported (Gillespie *et al.*, 2003a). Many of these aspects of the epidemiology of *Campylobacter* infections have recently been included in a

review by Humphrey *et al.* (2007).

Transmission Routes and Food-borne Sources

Infection with *Campylobacter jejuni* can be acquired from several different non food-borne sources. Transmission from water sources, either by drinking contaminated water or exposure during recreational use of environmental waters is well documented and there are many reports of outbreaks associated with water in the literature (Smith *et al.*, 2006). The most notable water-borne outbreak was associated with a municipal water supply in Walkerton, Canada (Clark *et al.*, 2003). From a food safety perspective water used to irrigate crops should not be contaminated with faecal pathogens and water used in food production should be of potable quality. Environmental contamination is a result of contamination from human and animal sewage. *Campylobacter jejuni/coli* do not multiply outside of the animal host but these organisms can survive long enough to result in exposure to infection. Even sand on bathing beaches can be contaminated with these pathogenic campylobacters and in a UK study between 30–60% of samples from beaches were contaminated (Bolton *et al.*, 1999). Direct contact with farm animals following school or educational visits is a known risk factor and contact with household pets, especially young dogs and cats with diarrhoeal illness (Evans, 1993). Unlike some other gastrointestinal infections person to person transmission is not a common feature.

Food-borne transmission either directly or indirectly is thought to be the most important route of infection and many food products have been associated with infection. Poultry, mainly chicken is known to be a major source of infection and is frequently contaminated with high numbers of organisms (Jorgensen *et al.*, 2002). *Campylobacter* carriage/colonisation in broiler flocks can be high and many poultry carcasses become contaminated during the slaughtering and evisceration process (Humphrey *et al.*, 2007). There have been many studies investigating methods to reduce contamination some with varying success (Anonymous, 2005). Although some countries *e.g.* Sweden have shown that it is possible to produce

Campylobacter free flocks (Anonymous, 2005). The main risk to consumers is the contamination of chicken sold at retail or used in catering. There have been many studies, around the world, and between 60–90% of retail chicken has been shown to be contaminated with campylobacters (Humphrey *et al.*, 2007). This can also be associated with contamination of the external packaging of retail products (Burgess *et al.*, 2005) which may then result in cross contamination of ready to eat foods.

Red meats are less frequently contaminated with campylobacters and studies have shown that about 2–6% of red meats sold at retail are contaminated (Humphrey *et al.*, 2007). The exception to this is raw meats such as offal, *e.g.*, liver and kidneys from food chain animals. Between 50–70% of samples have been shown to be contaminated with multiple types of *Campylobacter* species (Kramer *et al.*, 2000) and these food products have been associated with outbreaks of infection. Milk-borne transmission is also a source of infection, which is not surprising because raw cow's milk is intermittently contaminated with enteric pathogens. In a UK study 19/1097 (1.7%) samples of raw drinking milk was contaminated with *Campylobacter jejuni* (de Louvois and Rampling, 1998). Outbreaks of infection have been associated with drinking milk from cows and goats (Gillespie *et al.*, 2003 b; Hutchinson *et al.*, 1985). These have been in the main due to consumption of unpasteurised milk or following failure of pasteurisation at the dairy. Other food-borne sources include barbecued meat, salad vegetables (Humphrey *et al.*, 2007) and uncooked shellfish, particularly oysters.

One of the most important routes of transmission occurs during food handling and preparation in kitchens in both restaurants and in the home and at retail. Preparation of raw food, *e.g.*, chicken, can lead to cross contamination of other ready to eat food products. This was highlighted in a recent report from Japan (Yoda and Uchimura, 2006). Studies in the UK have shown that 12% of wiping cloths in Butcher's shops and 3% of cloths in retail food shops and in restaurants are contaminated with campylobacters (Bolton, F. J., personal communication). There is therefore a significant reser-

voir for cross contamination in these environments. Cross contamination is likely to be one of the major routes of transmission and can only be addressed by improving hygiene standards in kitchens and at retail.

Campylobacter jejuni/coli are susceptible to heating, pasteurisation, disinfectants and irradiation. (Humphrey *et al.*, 2007) These organisms can survive for over 7 days on poultry carcasses stored at refrigeration temperatures (Jorgensen *et al.*, 2002) Other aspects of the effect of low temperature storage, and response to pH, and desiccation have been reviewed by (Humphrey *et al.*, 2007)

Isolation of *Campylobacter jejuni* from Food Products

For growth and isolation of thermolerant campylobacters, including *C. jejuni* there are several parameters that must be optimised. These include temperature of incubation, microaerobic gaseous conditions and the choice of culture media.

C. jejuni grows between 32–45°C, although growth is poor at the extremes of this range and optimal growth occurs between 37–42°C. For isolation from food products several temperature combinations have been described. Ideally cultures should be pre-incubated at 37°C for 4–6 h to allow for recovery of sublethally damaged organisms followed by incubation at 41.5°C for 44–48 h. Incubation of enrichment broths and selective agar plates at 41.5°C has become universally accepted as the temperature of choice in international and national isolation protocols (ENISO, 2006; Health Protection Agency, 2005).

C. jejuni/coli are microaerophilic organisms and hence do not grow in air or under anaerobic conditions. Successful isolation requires gas mixtures containing about 5–10% oxygen, 5–10% carbon dioxide, 5–9% hydrogen in nitrogen (Bolton and Coates, 1983a). These microaerobic conditions can be achieved by either the evacuation-replacement technique using tank gas, gas generating sachets or by incubation in variable atmosphere incubators. There have been several comparisons and evaluations of these methods in the literature (Bolton and Coates, 1983b).

Campylobacter jejuni/coli grow poorly on

Table 1. Culture Media Supplements

Complex supplements	Defined supplements
Blood	Ferrous sulphate
Serum	Sodium metabisulphite
Charcoal*	Sodium pyruvate*
Oxyrase*	Haematin
	Sodium thioglycollate
	α -Ketoglutamic acid
	Catalase*

* Supplements that improve aerotolerance and aid recovery of sub-lethally injured campylobacters

basal agar media and therefore these basal peptones must be supplemented for successful isolation of these organisms from food and other sources (Bolton and Coates, 1983c). The basal media most frequently used in media for the isolation of campylobacters include: Nutrient broth No. 2, Brucella broth base, Blood agar base and Columbia agar (Corry *et al.*, 1995). These basal media are supplemented with either complex or defined supplements and sometimes both in combination. A list of supplements is given in Table 1. Complex supplements include blood and serum. Many of the culture media in use today still incorporate blood as the main supplement and blood is important in media formulations such as Skirrow agar, Butzler agar and Preston agar and broth (Corry *et al.*, 1995). Blood is a variable biological commodity and following investigation of the role of blood in *Campylobacter* media several complex and defined supplements were identified. (Bolton and Coates, 1983 c) For example the use of charcoal and oxyrase, which are complex supplements are more recent developments (Corry *et al.*, 1995).

Defined supplements include: ferrous sulphate, sodium metabisulphite and sodium pyruvate, known as the FBP supplement (George *et al.*, 1978). This defined supplement is particularly important because it improves aerotolerance of *C. jejuni* and detoxifies the effect of toxic peroxides and oxygen radicals which develop in culture media exposed to light (Bolton *et al.*, 1984a). The FBP supplement should therefore be added to all enrichment broths for the isolation of *C. jejuni* from food, water or environmental samples. A major breakthrough was the development of a blood free selective agar medium that uses charcoal as a supplement. Charcoal was shown to act in

Table 2. Antimicrobial selective agents used in *Campylobacter* culture media

Inhibitory to Gram negative organisms	Inhibitory to Gram positive organisms	Inhibitory to fungi and yeasts
Cephalosporins*	Vancomycin	Cyclohexamide
Trimethoprim	Teicoplanin	Amphotericin
Polymixin B/Colistin	Bacitracin	
Novobiocin	Rifampicin*	
	Sodium deoxycholate	

* Activity against both Gram negative and positive organisms

the same way as the FBP supplement and improves the aerotolerance and hence the recovery of *C. jejuni* (Bolton *et al.*, 1984a). Blood free media such as modified Charcoal, Cefoperazone, Deoxycholate agar (Bolton *et al.*, 1984b; Hutchinson and Bolton, 1984) and Karmali agar (Karmali *et al.*, 1986) are now widely used by food and clinical microbiologists. Corry *et al.* (1995), reviewed culture media for campylobacters and their excellent review is recommended for microbiologists interested in isolation of these important organisms.

Campylobacter jejuni/coli originate from the gastrointestinal tract of many animals and birds and therefore samples are always contaminated with many other different bacteria. Hence for successful isolation culture media must include selective agents to inhibit the growth of these unwanted organisms and to allow campylobacters to multiply. Many antimicrobial and antifungal agents have been used as selective agents in media for the isolation of campylobacters and the most important of these are listed in Table 2.

For successful isolation combinations of several selective agents are required. *Campylobacter* media incorporate selective agents that inhibit Gram positive organisms,

Gram negative organisms and fungi. The concentration of these selective agents must be determined for use with each different basal medium and for each combination to optimise recovery of *C. jejuni*. The use of mixtures of selective agents can create problems, for example the use of rifampicin and polymixin can prevent the isolation of sublethally damaged organisms. To overcome this effect it has been shown that delaying the addition of these selective agents benefits the recovery of *C. jejuni/coli*. (Humphrey, 1989). However successful isolation of campylobacters is a balance between selectivity and sensitivity and for the isolation from some type of heavily contaminated samples use of these selective agents is essential. A comprehensive list of *Campylobacter* enrichment broths and selective agars is presented in the review by Corry *et al.* (1995).

For the isolation of *C. jejuni* from food products the first stage is enrichment in a *Campylobacter* broth followed by plating onto a selective agar and examination for typical colonies. There have been many selective broth and agar formulations developed over the last 30 years in the past standard protocols have used Preston broth, Doyle and Roman broth or Park and Sanders broth in various combina-

Table 3. Formulation of Bolton broth*

Composition		Selective agents	
Meat peptone	10.0 g/L	Cefoperazone	20 mg/L
Lactalbumin hydrolysates	5.0 g/L	Vancomycin	20 mg/L
Yeast extract	5.0 g/L	Trimethoprim	20 mg/L
Sodium chloride	5.0 g/L	Cyclohexamide	50 mg/L
Sodium pyruvate	0.5 g/L		
α -Ketoglutamic acid	1.0 g/L		
Sodium metabisulphite	0.5 g/L		
Sodium carbonate	0.6 g/L		
Haemin	10.0 mg/L		
Lysed horse blood	50.0 ml/L		

* Bolton and Gibson (1994)

Fig. 1. Flow diagram of the Health Protection Agency (HPA) *Campylobacter* isolation procedure*

* Health Protection Agency (2005)

tions (Corry *et al.*, 1995). At the present time Bolton broth (Bolton and Gibson, 1994) has been adopted as the enrichment medium of choice for isolation from food samples. This medium was designed to resolve some of the issues described above and the formulation is given in Table 3.

Enrichment broths are used in conjunction with one or two of the following plating media: Modified Charcoal, Cefoperazone, Deoxycholate agar, Karmali agar or Skirrow agar. Food microbiologists are challenged with isolating campylobacters from many different types of samples and food matrices. To facilitate isolation standardised protocols have been developed as national methods (Health Protection Agency, 2005) or international methods (ENISO, 2006). A flow diagram of the current Health Protection Agency method is presented in Fig. 1. This method differs from the ENISO (2006) method in that microaerobic incubation of enrichment broths is not required and the enrichment broth is sub-cultured to a single plating medium. This protocol was the basis of the current ENISO method and both protocols can be used with all food products

Traditionally detection of campylobacters is achieved by isolating colonies on plates and confirmation using basic tests such as Gram stain, oxidase, motility, failure to grow aerobically and differentiation of *C. jejuni* from *C.*

coli is based on a positive hippurate test with *C. jejuni* isolates. However, there are now several commercially available immunoassays and PCR techniques that can be used to screen growth in enrichment broths. These techniques are likely to increase in popularity and be more widely adopted as the technology becomes more widespread. However enrichment broths will need to be optimised for these more rapid methods.

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