Campylobacter jejuni: An emerging pathogen

Nathanon Trachoo

Abstract
Trachoo, N.
Campylobacter jejuni: An emerging pathogen

Campylobacter jejuni is a major cause of food-borne diarrhea in many countries. However, in some countries, a number of cases were undetected because of the inappropriate detection method and ignorance. Although C. jejuni usually does not cause death in health adults, it can be deadly for immunocompromised persons (Pigrau, et al., 1997). Although thought to be very susceptible in several conditions, C. jejuni in fact is quite prevalent in nature. It can easily cause sporadic cases and outbreaks resulting in economic loss. This review covers three major parts: clinical aspects of Campylobacteriosis, C. jejuni reservoirs and transmission, and methods for detection.

Key words: Campylobacter jejuni, pathogen

Ph.D. (Food Science and Technology), Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand.
Corresponding e-mail: nathanon.t@msu.ac.th
Received, 11 September 2002 Accepted, 5 November 2002
Campylobacter jejuni and other Campylobacter species

At the beginning of 20th century, the microorganism, which was known as Vibrio fetus, was claimed to be responsible for spontaneous abortions in cattle and sheep. This organism differs from Vibrio species in that it cannot grow well under atmospheric oxygen tension and does not ferment sugars. In 1963, Sebald and Veron proposed the name Campylobacter in order to differentiate it from Vibrio species (Catteau, 1995). Some scientists believe that Escherich, a German physician, observed Campylobacter organisms in the stool of infants with diarrhea in 1880.

There are 11 species in the genus Campylobacter including C. fetus, C. hyointestinalis, C. sputorum, C. jejuni, C. coli, C. lari, C. upsaliensis, C. mucosalis, C. concisus, C. curvus, and C. rectus. Isolation of Campylobacter organisms was not successful until 1972 when Dekeyser (1972) isolated Campylobacter by culturing filtered C. jejuni on a blood-thioglycolate agar medium containing bacitracin, polymyxin B sulfate, novobiocin, actidione. C. jejuni is the most commonly identified species (Nachamkin, et al., 1992). C. jejuni was formerly named C. fetus subsp. jejuni. It differs from the other species in that it is hippurate positive (Catteau, 1995).

Bergey’s Manual of Determinative Bacteriology (Holt, et al., 1994) describes C. jejuni as "Slender, vibrioid cells, 0.2 to 0.5 µm wide and 0.5-5 µm long. The rods may have one or more helical turns and can be as long as 8 µm. They also appear S-shaped and gull-wing-shaped when two cells form short chains. They are non-sporeforming. Cells in the old cultures may form spherical forms (coccoid bodies). Stain is Gram-negative. Cells are motile with a characteristic cork-screw-like motion by means of a single, unsheathed, polar flagellum at one or both ends of the cells. They are typically microaerophilic and have a respiratory type of metabolism. They typically require an O₂ concentration between 3-15% and a CO₂ concentration of 3-5%.”

Clinical aspects

C. jejuni and C. coli are both responsible for Campylobacter enteritis but C. jejuni infections are more prevalent. Campylobacter enteritis affects mostly children under the age of 5. In most cases, the incubation period is 2 to 5 days. The sickness begins with a fever associated with malaise and headaches followed with nausea and abdominal cramping resembling the symptoms of acute appendicitis (Altekruse, et al., 1999, Catteau, 1995). Patients in developing countries appear to have less severe symptoms than those in developed countries. In Thailand, only one third of symptomatic infections were bloody while half of the
Patients with Campylobacter enteritis in the United States had bloody diarrhea (Nachamkin, et al., 1992). Occasionally, C. jejuni infections lead to bacteremia, septic arthritis, and other complications. C. jejuni is believed to be an opportunistic organism in immunocompromised patients. For instance, a surveillance report from Los Angeles County during the year 1983 to 1987 indicated the rate of Campylobacter enteritis among patients with acquired immune deficiency syndrome (AIDS) was 39 times greater than that in general population (Sorvillo, et al., 1991). Common complications of Campylobacter infection are persistent infection and infection with antimicrobial resistant strains of campylobacters in AIDS patients (Perlman, et al., 1988). C. jejuni infection rarely results in death, which only occurs in children and immunocompromised persons. Usually Campylobacter enteritis is self-limiting (Blaser, 1997). However antibiotic treatment may be used in patients with high fever, bloody diarrhea, or diarrhea of more than eight stools in one day, and immunocompromised patients (Altekruse, et al., 1999). Erythromycin has been the drug of choice for treatment of Campylobacter infections because of its ease of use, high degree of efficacity, and lack of serious toxicity. Early antibiotic treatment has been studied by several groups (Salazar-Lindo, et al., 1986, Schwartz, 1987, Williams, et al., 1989) and is effective.

Recently, C. jejuni has been linked to Guillain-Barre Syndrome (GBS). This disease is characterized by rapid progressive symmetric paralysis. Although the trigger for this immune attack is not known, GBS is often preceded by Campylobacter infection (Walk, 1997). Approximately, one case of GBS occurs for every 1000 cases of Campylobacteriosis (Altekruse, et al., 1999). O:19 strains was the most common serotype among GBS associated C. jejuni strains. Allos et al. (1998) suggested that the insensitivity of these strains to lytic effects of complement allows them to trigger delicate specific immunologic response.

C. jejuni outbreak and infection cases

Although some scientists believe that most Campylobacter infection cases are sporadic, Campylobacter outbreaks have been reported from around the world (Alary and Nadeau, 1990, Anderson, et al., 1997, Itoh, et al., 1980, Millson, et al., 1991, Rautelin and Hanninen, 2000, Taylor, et al., 1991). The first reported C. jejuni outbreak in the United States was in 1978 in Bennington, Vermont. Approximately, 3000 people, 19% of the population, were infected by C. jejuni contaminated water. This appears to be the largest C. jejuni outbreak in the U.S. (Vogt, et al., 1982). Fifty-seven outbreaks of C. jejuni were reported between 1978 and 1986. Although water-borne Campylobacter outbreaks occur less frequently, they are more severe (Nachamkin, et al., 1992).


Campylobacter outbreaks occasionally occur in conjunction with other pathogens such as
Antimicrobial resistance

Since C. jejuni was recognized as a pathogen, reports on its antimicrobial resistance have increased (Naeeem and Macaulay, 1983). This may be due to the extensive use of selected antimicrobial agents in human and food animals (Aarestrup and Wegener, 1999, Harnett, et al., 1995, Piddock, 1995). The emergence of fluoroquinolones resistant Campylobacter coincides with the increasing use of fluoroquinolones in human and veterinary medicine (Bowler, et al., 1996, Endtz, et al., 1991). Research suggested that fluoroquinolones such as ciprofloxacin, temafloxacin, and norfloxacin may now be of limited use in the treatment of Campylobacter infections in some regions (Engberg, et al., 2001). In Taiwan, the prevalence of macro-lide and quinolone resistant Campylobacter isolates has increased during the past decade (Lu, et al., 2000). Pigrau and coworkers (1997) reported that antibiotic resistance rates of Campylobacter species isolated from 58 patients in a hospital in Spain were 82% for cephalothin and 79% for cotrimoxazole. According to a study in Norway, resistance of Campylobacter jejuni to erythromycin is very rare and therefore it is recommended as the drug of choice in Norway (Afset and Macelisland, 2001). Campylobacter resistance to antimicrobial agents has changed with time (Ruiz, et al., 1998) indicating that this change may be associated with the changes in antibiotic usages. However, research shows that mutation associated with clinically significant resistance to the quinolones in C. jejuni occurs in vivo (Segreti, et al., 1992, Wretlind, et al., 1992). C. coli, another Campylobacter organism less frequently causing Campylobacter enteritis, was reported to be resistant to erythromycin, ampicillin, gentamicin, and amikacin (aenz-Y, et al., 2000); while C. jejuni was less resistant to these antibiotics (Lekowska-Kochaniak, et al., 1996). Antibiotic resistance in campylobacters may or may not be plasmid-mediated (Bradbury and Munroe, 1985, Lekowska-Kochaniak, et al., 1996, Velazquez, et al., 1995).

C. jejuni reservoirs and transmission

C. jejuni is sensitive to oxygen tension (Hoffman, et al., 1979), ambient temperature (Blaser, et al., 1980), and drying (Doyle and Roman, 1982). Transmission vectors play a major role in the epidemiology of this pathogen. Despite its fastidious nature, C. jejuni has multiple sources. Unsurprisingly, numerous cases of Campylobacter infection went recognized due to lack of proper detection methods in the past. Wild and domestic animals potentially harbor the pathogen (Hald and Madsen, 1997, Weber, 1985). Rosef et al. (1981) investigated the occurrence of C. jejuni in a total of 129 wild birds consisting of 71 pigeons, 54 seagulls, three crows, and one raven and found that 24.8% of these birds carried C. jejuni. Close contact with infected animals is a significant cause of campylobacteriosis in children (Kapperud, et al., 1992, Luechtefeld, et al., 1981, Shane and Montrose, 1985, Svedhem and Kaijser, 1981). Migratory birds that carry C. jejuni may also shed the pathogen into lakes and rivers (Luechtefeld, et al., 1980). A report (Ellis, et al., 1995) showed that Campylobacter infection is an occupational disease among farm workers. This may be due to the close contact of the workers with farm animals.

Water is often a source of C. jejuni (Arvanitiou, et al., 1995, Bolton, et al., 1987). Forty two cases of Campylobacter infections in Christchurch, New Zealand resulted from drinking contaminated water (Stehr-Green, et al., 1991). Another study showed that dairy cows were infected by
C. jejuni in lake water (Hanninen, et al., 1998). The control animals, which were given chlorinated tap water, had a lower infection rate. C. jejuni often could not be isolated from water samples by using a conventional method (Hill and Grimes, 1984). Reports showed that this organism can be viable but nonculturable (VBNC) (Jones, et al., 1991, Rollins and Colwell, 1986). Occurrence of C. jejuni in water may be seasonal (Carter, et al., 1987). The recovery rates of C. jejuni were highest in the fall and winter months and lowest during the spring and summer months. The report indicated that microbiological (plate counts of fecal and total coliforms, fecal streptococci, and heterotrophic bacteria) or physical (water temperature, pH, and conductivity) factors did not have significant effects on the occurrence of C. jejuni. C. jejuni has been isolated by filter enrichment, a nonconventional technique, from groundwater (Stanley, et al., 1998). VandeGiessen et al. (1996) suggested that groundwater was the source of C. jejuni infection in broiler chickens.

Poultry foods are recognized as a major vehicle for transmission of C. jejuni infection. The prevalence of C. jejuni on poultry foods was extensively reviewed by Bryan and Doyle (1995). Common poultry products associated with C. jejuni are uncooked chicken parts, chicken organs (livers, hearts, gizzards), chicken wings, and turkey. It was only in the early of 1980s that C. jejuni was recognized as a major contaminant in raw poultry products along with Salmonella (Norberg, 1981). This may be due to lack of suitable methodology for isolation. Occurrences of C. jejuni in poultry foods have been reported worldwide. Research conducted in Japan showed that C. jejuni was found in domestic retailed poultry (45.8%), but not in beef or pork. In the poultry processing plant, chicken carcases, equipment, and workers hands were contaminated with C. jejuni (Ono and Yamamoto, 1999). Roasted chicken tacos in Mexico City were found to be contaminated with Campylobacter organisms, especially, C. jejuni as a result of cross-contamination (Quinones-Ramirez, et al., 2000). Enteropathogenic campylobacters (C. jejuni and C. coli) were found on 68% of whole chickens, 100% of chicken parts, and 100% of organs from retail markets in Taipei, Taiwan (no refrigeration was available) (Shih, 2000).

Other reservoirs for C. jejuni include insects, pets, feral animals, and porcine carriage (Stern, 1992). Seafoods were associated in two outbreaks (Stern, 1992). It is possible that the seafood was from water contaminated with C. jejuni.

**Survival of C. jejuni in aquatic systems**

Despite its susceptibility to atmospheric oxygen and inability to grow at ambient temperature, C. jejuni has been detected in natural aquatic environments including river water, ground water, coastal water, and lake water (Arvanitidou, et al., 1995, Bolton, et al., 1982, Chynoweth, et al., 1998, Hanninen, et al., 1998). Wastewater has also been reported to be a source of contamination of C. jejuni (Stelzer, et al., 1988). A two-year joint study by the U.S. Central Disease Control (CDC) and the U.S. Environmental Protection Agency showed that Campylobacter jejuni was responsible for three waterborne outbreaks during 1993-1994 (Kramer, et al., 1996). Bolton et al. (1987) observed that river waters contain a variety of Campylobacter spp. and that river water is a potential source of C. jejuni. Its survival strategies in various aquatic environments will be reviewed.

C. jejuni in the aquatic environment is metabolically different from laboratory-grown C. jejuni. C. jejuni in various aquatic systems and at various temperatures (4, 10, 22, and 37°C) has been found to lose culturability on conventional media after certain period of time (Buswell, et al., 1998). The survival of C. jejuni has been tested in drinking water, river water, and sewage (Pickert and Botzenhart, 1985) and reported that C. jejuni could only survive for a few days in the waters. This study also showed that the concentration of oxygen or nutrients in the water did not affect the survival of C. jejuni. Another study using a nalidixic acid elongation method showed that C. jejuni could survive in stream water up to 4 months at
4°C (Rollins and Colwell, 1986). However, survivability of *C. jejuni* depends greatly on strains (Buswell, et al., 1998).

**Significance of viable but nonculturableView (VBNC) forms**

Recently, strong evidence has supported the existence of VBNC *C. jejuni* in aquatic environments (Jones, et al., 1991, Rollins and Colwell, 1986, Talibart, et al., 2000, Tholozan, et al., 1999). The transformation of spiral to coccal shape has been implied to be associated with oxidative stress of *C. jejuni* in high oxygen-tension environments including atmospheric oxygen tension (Harvey and Leach, 1998). This change occurs in conjunction with reduction in maximum growth rate and viable count (Harvey and Leach, 1998). VBNC may be a degenerate form of *C. jejuni* resulting from oxidative stress. Ability to maintain differences between internal and external pH values of VBNC *C. jejuni* is reduced with time of incubation in microcosm water (Tholozan, et al., 1999). However, the immediate consequence may not be death. Federighi (1998) demonstrated that VBNC *C. jejuni* can revert to a culturable pathogenic state after 30 days of incubation in microcosm water. The resuscitated *C. jejuni* are capable of infecting suckling mice but this property may differ between strains (Jones, et al., 1991). VBNC *C. jejuni* are also capable of colonization in the yolk sacs of embryonated eggs (Cappelier, et al., 1999). A few research groups have studied colonization of VBNC *C. jejuni* in chickens. Stern and coworkers (1994) reported that only some isolates of VBNC *C. jejuni* (four out of six) were capable of colonization in 5 of 79 chicks. The infectious oral dose of freshly cultured cells of this model was 26-260 cfu. Medema and coworkers (1992) studied the ability of VBNC *C. jejuni* to colonize the intestine of day-old chicks. When VBNC *C. jejuni* (1.8 x 10^5 cfu/ml) were administered to day-old chicks orally *C. jejuni* was not isolated from embryonated eggs or the caeca of the chicks after incubation for 7 days.

*C. jejuni* has been isolated from a wide variety of sources including the aquatic environment, wildlife, insects, and domestic animals. *C. jejuni* in these settings are likely to be sublethally injured, metabolically reduced, VBNC or completely inactive. They may be also present in a very low number. The works of Stern et al. (1994) and Medema et al. (1992) suggest that *C. jejuni* colonization occurs randomly in some young chicks by metabolically active *C. jejuni* from those sources. Only a few infected chickens can spread *C. jejuni* to the entire flock (Hingley, 1999) in a rapid rate via fecal contamination and water. This chicken-to-chicken transmission appears to be effective because *C. jejuni* cells have less time to be out in the environment until being ingested by another host. Control of this type of transmission may be very difficult.

**Water biofilms as campylobacters reservoirs**

Water biofilms may contain various human pathogens including coliforms, Salmonella, and *Legionella pneumophila* (Berger, et al., 1992, Mattila-Sandholm and Wirtanen, 1992). *C. jejuni* has also been isolated from water biofilms in ground water (Stanley, et al., 1998). Aquatic biofilms enhance survival of some pathogens in natural settings including *Legionella* species and *Salmonella* (Barker and Bloomfield, 2000, Lee and West, 1991, Payment, et al., 1988). There has been little study on the influence of aquatic biofilm on survival of *C. jejuni*. Trachoo and coworkers (2002) reported that biofilms isolated from chicken house enhanced the survival of *C. jejuni* during incubation at 12 and 23°C over a 7-day period. Buswell and coworkers (1998) studied extended survival of *C. jejuni*, *C. coli*, and *C. upsaliensis* with autochthonous water microflora (biofilm) grown on glass slides. They compared the survival times of *C. jejuni* in treatments with and without biofilms and found that the biofilms enhanced the survival of *C. jejuni* from 230 h to 700 h for strain CH1 and 157 h to 360 h for strain 9752. They also investigated the effect of strain differences on survival of *C. jejuni* under various
temperatures and oxygenations and found that survival times of *C. jejuni* depend greatly on strains. Oxygen concentration within biofilm varies greatly depending on the depth of biofilm (Costerton, *et al.*, 1995, Costerton, *et al.*, 1994). Biofilm near substratum surface has lower oxygen tension, which may be less harmful for *C. jejuni*. Extracellular polysaccharides (EPS) are produced by the microorganisms in the biofilm, which may also increase the attachment of nearby microorganisms and provide protection against chemical sanitizers in the water supply system (Berger, *et al.*, 1992, Thiel, *et al.*, 1993). Effectiveness of chlorine, peracetic acid, peroctanoic acid and quaternary ammonium compounds in inactivation of *C. jejuni* in biofilm isolated from chicken house has been determined. Chlorine was the most effective sanitizer for inactivation of *C. jejuni* and the quaternary ammonium compounds was the least effective. Authors suspected that chlorine may be less effective in system with more organic materials (Trachoo and Frank, 2002).

**Aerotolerance**

Two reports have shown that microaerophilic *C. jejuni* can adapt to aerobic growth. After being left in air for 2-3 days, all strains used in the study conducted by Jones and coworkers (1993) grew freely in air on subculture and could be further subcultured indefinitely in air. This adaptation occurs in conjunction with a colony morphology change and some changes in outer membrane protein patterns. The individual cell morphology and ability to colonize mice remained unchanged in the air-adapted cells. They also reported that the air-adapted *C. jejuni* retained its ability to grow in air even after recovery from mouse gut. Additional human isolates of *C. jejuni* were found to adapt to aerobic growth by Chynoweth *et al.* (1998). Forty isolates from patients were tested for air adaptation ability. About 72% could be adapted to grow on nutrient agar under aerobic conditions. The study also suggested that the air-adapted *C. jejuni* survived as well as the microaerophilic *C. jejuni* at 5 and 25°C. However, the difference occurred at 37°C when the microaerophilic *C. jejuni* persisted for a longer time. The adaptation did not enhance aerobic survival. These findings indicate a potential for some strains of *C. jejuni* to survive in the aerobic environments.

**Stresses associated with temperature**

Pickert and Botzenhart (1985) suggested that temperature significantly affects survival of *C. jejuni*. Buswell and coworkers (1998) demonstrated the different survival times of 17 different strains of campylobacters. Generally, lower temperatures preserved viable *C. jejuni* cells. Means of persistence time at 4, 10, 22, and 37°C were 201.6, 175.8, 42.6, and 21.8 h, respectively.

**Methods for detection of *C. jejuni***

**Conventional methods**

Under optimal growth conditions, *C. jejuni* can reach $10^7$-10$^8$ cfu/ml in a laboratory media but also dies off rapidly in the air or becomes less active at ambient temperature (Catteau, 1995). Most strains require an oxygen concentration of 3-6% for growth, usually 5% O$_2$, 10% CO$_2$, and 85% nitrogen (Catteau, 1995). Oxygen tolerance of *Campylobacter* depends greatly on the species and strains (Chynoweth, *et al.*, 1998, Stern, 1982). Luechtefeld *et al.* (1982) compared the effect of the conventional atmosphere of 5% oxygen and 8% carbon dioxide with a candle jar (17% oxygen and 3% carbon dioxide) on isolation of *C. jejuni* from 263 positive canine, cattle and turkey fecal or cecal specimens. They found that at an incubation temperature of 42°C, the conventional atmosphere with 5% oxygen resulted in more *C. jejuni* colonies per plate with consistently larger colonies (Luechtefeld, *et al.*, 1982). Wang *et al.* (1982) demonstrated that the use of a candle jar was suitable for primary isolation of *C. jejuni* from 263 positive canine, cattle and turkey fecal or cecal specimens. They found that at an incubation temperature of 42°C, the conventional atmosphere with 5% oxygen resulted in more *C. jejuni* colonies per plate with consistently larger colonies (Luechtefeld, *et al.*, 1982). They also compared effectiveness of Campak II$^{	ext{TM}}$ (BBL Microbiology Systems, Cockeysville, Md.) and the conventional 5% oxygen for the 6-plate per jar system at 42°C and found no significant differences.

The microaerophilic nature of *C. jejuni* has complicated its recovery from human and animal
sources. The addition of reducing agents to cultural media greatly enhances the recovery of this organism. Ferrous sulfate, sodium metabisulfate, and sodium pyruvate (FBP) enhance the growth of C. jejuni in both supplemented agar and broth (George, et al., 1978). However, Luechtefeld et al. (1982) reported that FBP enhanced the growth of C. jejuni at 42°C but not at 37°C. The mechanism of these reducing agents is not understood they may quench toxic superoxide anions (Hoffman, et al., 1979). Histidine was reported to quench singlet oxygen in photo-oxidized media, therefore enhancing the growth of C. jejuni (Juven and Rosenthal, 1985). Sodium dithionite and histidine added to nutrient agar allows C. jejuni to grow in air. The addition of superoxide dismutase, a hydroxyl radical scavenger such as cysteamine, or the free radical antioxidants like tocopherol and butylated hydroxy toluene, did not increase the recovery rate of photochemically injured cells (Juven and Rosenthal, 1985). Although C. jejuni can grow on blood-free media, its presence promotes the growth of C. jejuni (Barot and Bokkenheuser, 1984, Gericke, et al., 1988).

Since C. jejuni are present in the environment and foods in a very low number, suppression of other microorganisms greatly improves the detection and recovery. C. jejuni is resistant to several antibiotics including vancomycin, polymyxin B, trimethoprim lactate, and the cephalosporins. These antibiotics are often included in selective media of C. jejuni. Table 1 shows the target organisms of these antibiotics. Excessive addition of antibiotics can adversely affect the growth of C. jejuni (Ng, et al., 1985).

Methods for detection of C. jejuni were originally developed for clinical specimens. Slight variations have been added to detection methods of C. jejuni from foods, especially the addition of an enrichment step. Enrichment is not necessary for clinical specimens such as feces collected from patients with diarrhea. This was demonstrated by Gilchrist (1981) and Agulla et al., (1987). However, enrichment is required for C. jejuni isolation from foods and environmental samples. This is because C. jejuni are present in these samples in a very low number with high level of competing microflora (Stern, 1992). In addition to enrichment, isolation media used for C. jejuni in foods and environmental samples should contain oxygen quenchers and antibiotics. Incubation under microaerophilic condition is also recommended (Stern, 1982). Detection and isolation of C. jejuni in water may present some problems (Pokorny, 1990). Rollins and Colwell (1986) observed VBNC state of C. jejuni in stream water held at 4°C. Filtration of water samples may be required for detection of C. jejuni in aquatic environments (Mathewson, et al., 1983, Oyofo and Rollins, 1993). Viability staining has been employed for C. jejuni detection. Tholozan et al. (1999) used 5-cyano-2,3-ditolyl tetrazolium chlo-

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>Gram positive cocci</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Enterobacteriaceae, Pseudomonas sp.</td>
</tr>
<tr>
<td>Trimethoprim lactate</td>
<td>Proteus sp.</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Streptococcus faecalis, Enterobacter sp.</td>
</tr>
<tr>
<td></td>
<td>Yersinia enterocolitica, Serratia sp.</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa and Bacteroides fragilis.</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Yeasts and fungi</td>
</tr>
<tr>
<td>Cycloheximide (actidione)</td>
<td>Yeasts and fungi</td>
</tr>
</tbody>
</table>

Modified from Stern (1982)
raine-4, 6-diamino-2-phenylindole to detect VBNC C. jejuni in microcosm water. Stained and elongated cells of viable C. jejuni resulting from incubation with nalidixic acid can be observed under a microscope (Federighi, et al., 1998). Nalidixic acid is a DNA gyrase inhibitor preventing cell multiplication or more precisely cell wall separation (Kogue, et al., 1979). Viable C. jejuni cells can be detected by using a double staining technique (Cappelier, et al., 1997, Federighi, et al., 1998). First C. jejuni cells are stained with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) resulting in formazan deposition in cells. CTC is reduced in an electron transport system of respiring cells to an insoluble red fluorescent formazan (Rodriguez, et al., 1992). The cells are then counter stained with 4′-6 diamino-2-phenylindole (DAPI), which fluoresces in blue. This method allows simultaneous enumeration of total and viable bacteria on a single filter. Cappelier (1997) recommended four hours of incubation with 5 mM CTC under a microaerobic atmosphere. Direct fluorescent antibodies (DFA) have been used for C. enteritis rapid screening (Hodge, et al., 1986). The use of antibodies allows specific identification of C. jejuni in the presence of other normal microflora. Buswell (1998) tested an indirect fluorescent antibody method (IDFA) for detection of C. jejuni attached on glass slides containing aquatic biofilms. Nonspecific fluorescence was observed. They suggested that the biofilms may express cross-reactive epitopes and the biofilm matrix may be coated with the reactive epitopes from C. jejuni through cell lysis or excretion.

**Molecular based methods**

Due to prevalence of C. jejuni in Campylobacter enteritis in human, rapid detection methods for C. jejuni have been developed. Immunostaining can be rapid but non-specific binding of antibodies can occur. Enzyme linked immunosorbent assay (ELISA) has been used as a detection method for C. jejuni in stools of patients with Campylobacter enteritis (Nakamura, et al., 1986). DNA-DNA hybridization has successfully been used to detect Salmonella sp. in foods with the requirement of non-selective enrichment (Fitts, et al., 1983). This technique can also be used for sensitive detection of food-borne enterotoxigenic E. coli in artificially contaminated foods without enrichment (sensitivity level of 100 cells per g) (Hill, et al., 1983). A direct colony DNA-DNA hybridization method was used for the detection of C. jejuni in chicken feces and it was sensitive enough to detect a small number (100 cfug/mL) of C. jejuni in chicken feces, which contained a large number of background flora (Chuma, et al., 1993). A major problem in detecting C. jejuni in foods is that indigenous flora are present in a much greater number than C. jejuni. An assay to detect C. jejuni in foods that uses a short selective enrichment followed by nucleic acid sequence-based amplification (NASBA) amplification of RNA and a nonradioactive in-solution hybridization was studied (Uyttendaele, et al., 1995). Detection of C. jejuni was successful up to a ratio of indigenous flora to C. jejuni of 10,000:1. The use of enrichment culture and NASBA shortened the analysis time from 6 days to 26 h.

Polymerase Chain Reaction (PCR) has been used as a method for detection of several food-borne pathogens including E. coli (Caiero, et al., 1999, Wermars, et al., 1991), Shigella flexneri (Lampel, et al., 1990), Staphylococcus aureus (Khan, et al., 1998), and C. jejuni (Waegel and Nachamkin, 1996). This method is based on the activity of Taq polymerase at a DNA annealing temperature of 60°C, which allows first duplication, and later amplification of target DNA sequence found in a pathogen of interest (Lodish, et al., 1999). Stonnet et al. (1995) successfully detected tested all the C. jejuni and C. coli strains without any false positive or false-negative by using a PCR method. Flagellin gene is often used as target sequence for detection of C. jejuni and C. coli in PCR methods resulting in a high level of sensitivity and specificity (Comi, et al., 1995). The PCR method has higher level of sensitivity (approximately 25 cfug/mL) than DNA probes based methods (10^6 to 10^7 cfug/mL) (Giesendorf, et al., 1992). A highly sensitive (1 cell/ml) and rapid method
based on PCR was developed by Waller and Ogata (2000). The quantitative method allows direct detection of the pathogen without an enrichment step and results can be obtained in approximately 8 h. Some foods present problems when using PCR to detect C. jejuni. This may be because they contain some substances that inhibit Taq polymerase reaction. A study conducted by Winters and coworkers (1998) demonstrated this potential problem. Although most foods are compatible with the PCR method, apple and strawberries gave a false negative reaction (Winters, et al., 1998). A multiplex PCR, which allows several DNA segments to be amplified by using multiple pairs of primers (Atlas and Bej, 1994), has been developed for C. jejuni genotypic characterization (Ragimbeau, et al., 1998). Reverse transcriptase enzyme can be used to synthesize a complementary DNA (cDNA) of target RNA before the PCR is begun (Atlas and Bej, 1994). Messenger RNA (mRNA) molecules, a requirement for cellular protein synthesis, have a short half-life (Lodish, et al., 1999). The presence of mRNA therefore indicates viability. Reverse transcriptase PCR (RT-PCR) can be used to detect viable cells while PCR would detect both dead and live cells. RT-PCR has been used as a rapid detection method for several food-borne pathogens for some time. Sheridan and coworkers (1999) used PCR and RT-PCR to detected live and dead (killed by autoclaving, boiling, and 50% ethanol) of E. coli. They found that PCR was positive in both but RT-PCR was positive only in healthy cells. Recently, this method was developed for the detection of viable enteropathogenic campylobacters (Sails, et al., 1998). The assay is specific for C. jejuni, C. coli and C. upsaliensis. The restriction endonucleases, Alu I, Dde I, and Dra I, were used to obtain restriction fragment of the 256 bp. This assay was used to detect C. jejuni cells (heated at 72°C for 5 min) and mRNA was detected by RT-PCR immediately after heat killing but became undetectable within 4 h when the cells were held at 37°C. Although claimed to be able to differentiate between live and dead cells of C. jejuni, immediate detection of freshly killed cells may result in false positive. This is mainly due to the fact that mRNA does not degenerate immediately after the cells are inactivated.

Conclusions

C. jejuni causes food infection worldwide and this indicates the needs for intervention and prevention. Increased use of antibiotics in animals and humans at alarming rate may cause more antimicrobial resistant strains of C. jejuni. The problem in preventing C. jejuni contamination in the food chain is that this organism is quite prevalent and can persist some adverse conditions in the nature for extended period of time. Several research groups are developing rapid and high accuracy detection methods for C. jejuni. This will give new hopes for the food and animal production sectors to early detect the cause of infection. However, a good detection method cannot be the only tool for elimination of the food infection. Educational media can be used to increase the public awareness and understanding, which is the better tool for prevention of any foodborne illnesses.

References


Campylobacter jejuni: An emerging pathogen

Trachoo, N.

Vol. 25 No. 1 Jan.-Feb. 2003


Campylobacter jejuni: An emerging pathogen

Trachoo, N.

Vol. 25 No. 1 Jan.-Feb. 2003

152


Microbiol., 8:36-41.


Campylobacter jejuni: An emerging pathogen

Trachoo, N.

Vol. 25 No. 1 Jan.-Feb. 2003


Campylobacter jejuni: An emerging pathogen


