REVIEW ARTICLE

Campylobacter jejuni : An emerging pathogen

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Abstract

Trachoo, N.
***Campylobacter jejuni* : An emerging pathogen**
Songklanakarin J. Sci. Technol., 2003, 25(1) : 141-157

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

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Received, 11 September 2002 Accepted, 5 November 2002

บทคัดย่อ

ณฐนนท์ ตราชู
Campylobacter jejuni **: เชื้อโรคตัวใหม่**
ว. สงขลานครินทร์ วทท. 2546 25(1) : 141-157

Campylobacter jejuni เป็นสาเหตุสำคัญของโรคท้องร่วงเนื่องมากจากอาหารในหลายประเทศ ตัวเลขของผู้ป่วยติดเชื้อในบางประเทศอาจจะต่ำกว่าความเป็นจริงเนื่องจากขาดวิธีการตรวจนับเชื้อที่เหมาะสมและละเลยในการหาสาเหตุของโรค แม้ว่า ***Campylobacter jejuni*** จะไม่ทำให้คนปกติที่ติดเชื้อต้องถึงแก่ชีวิต แต่อาจทำให้เด็กและผู้ที่มีภูมิคุ้มกันต่ำป่วยจนเสียชีวิตได้ เชื้อ ***Campylobacter jejuni*** ไม่สามารถเจริญได้ในหลายสภาพแวดล้อม แต่ก็มีรายงานเสมอว่าตรวจพบเชื้อนี้ในธรรมชาติ และเป็นสาเหตุให้เกิดโรคระบาดและการล้มป่วย บทความนี้ครอบคลุมเนื้อหาหลัก 3 ประเด็นคือ โรคที่เกิดจาก ***Campylobacter jejuni*** แหล่งและการติดต่อของเชื้อ และการตรวจนับเชื้อ

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C. 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, vibriod 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 O_2 concentration between 3-15% and a CO_2 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 afflicts 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 to1987 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 efficacy, 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 are associated with milk, raw poultry, water, and ground water (Alary and Nadeau, 1990, Evans, *et al.*, 1998, Klein, *et al.*, 1986, Shandera, *et al.*, 1992, Stehr-Green, *et al.*, 1991, Vogt, *et al.*, 1982). Fahey *et al.* (1995) investigated an outbreak of gastrointestinal illness affecting at least 110 people, of whom 41 had microbiological confirmation of *C. jejuni* infection. They concluded that the outbreak was associated with under pasteurized milk. Water-borne *Campylobacter* outbreaks affect people on a large scale ranging from a few hundred to a few thousand cases (Alary and Nadeau, 1990, Millson, *et al.*, 1991, Vogt, *et al.*, 1982). Improperly sanitized water was often responsible for the water-borne *Campylobacter* outbreaks (Millson, *et al.*, 1991). Chicken and turkey have been linked to *C. jejuni* outbreaks (Murphy, *et al.*, 1995). Evans *et al.* (1998) investigated a *C. jejuni* outbreak, which caused by consumptions of stir-fried food. They suggested that undercooked chicken in the stir-fried was the cause of the outbreak. Often the foods responsible for outbreaks did not contain poultry, but *C. jejuni* was cross contaminated from raw poultry prepared in the same kitchen (Anonymous, 1998, Brown, *et al.*, 1988, Roels, *et al.*, 1998).

Campylobacter outbreaks occasionally occur in conjunction with other pathogens such as

Escherichia coli O157 (Jones and Roworth, 1996), *Salmonella* (Layton, *et al*., 1997), and *Cryptospodium* (Duke, *et al*., 1996). This indicates the prevalence of this organism. *Campylobacter* outbreaks commonly occur in public facilities including schools, daycare centers, nursing homes, training facilities, and hospitals (Brown, *et al*., 1988, Itoh, *et al*., 1980, Kirk, *et al*., 1997, Layton, *et al*., 1997, Murphy, *et al*., 1995, Stuart, *et al*., 1997, Yanagisawa, 1980). This is mainly due to poor sanitation and cross contamination during food preparation.

Antimicrobial resistance

Since *C. jejuni* was recognized as a pathogen, reports on its antimicrobial resistance have increased (Naeem 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 macrolide 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 cotrimoxazone. 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 Maeland, 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* (Arvanitidou, *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 carcasses, 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 nonculturable (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* (1.8×10^5 *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 human feces at 42°C, but not at 37°C. They also compared effectiveness of Campak II™ (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 quenche 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, polymycin 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 ori-ginally 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 1. Inhibitory effect of antibiotics used as selective agents for *C. jejuni*

Antibiotics	Targets
Vancomycin	Gram positive cocci
Polymyxin B	*Enterobacteriaceae, Pseudomonas* sp.
Trimethoprim lactate	*Proteus* sp.
Cephalosporins	*Streptococcus faecalis, Enterobacter* sp, *Yersinia enterocolitica, Serratia* sp, *P. aeroginosa* and *Bacteroides fragilis*.
Amphotericin B	Yeasts and fungi
Cyclohexmide (actidione)	Yeasts and fungi

Modified from Stern (1982)

rine-4', 6-diamino-2-phenylinedole 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 cfu/g) 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* (Caeiro, *et al.*, 1999, Wernars, *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 *cfu*/g) than DNA probes based methods (10^5 to 10^7 *cfu*/g) (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 PRC (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.

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