# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Microbiology and taxonomy of *Campylobacter spp.*

Because of the increasing interest in *Campylobacter*-like organisms and the rapid development of new technology, the taxonomy of *Campylobacters* and other related bacteria has gone through drastic changes. The history of taxonomy began since 1886, when the non-cultural spiral-shaped bacteria were observed the association with enteritis in neonates, infants and kittens. These organisms can only be detected under the microscope because of their failure to grow on solid medium. During the 19$^{th}$ century, these organisms were successfully isolated from aborted ovine and bovine fetuses or blood from aborted women, which was reported as *Vibrios*-like organisms. Then the causing organism of this infectious infertility was named *Vibrio fetus* subsp. *Venerealis* and *V.fetus* subsp. *intestinalis*, which are currently known as *Campylobacter fetus* subp. *venerealis and C.fetus* subsp. *fetus*, respectively.

In the following decades, *V.jejuni* was identified from bovine dysentery and found a causal relation with microaerophilic *Vibrios*. Similar to organisms detected in blood cultures of humans gastroenteritis were associated with those

aborted sheep fetuses. Diarrhea pigs were also found *V.coli*. While *V.sputorum* was isolated from sputum of bronchitis patients known to be *C.sputorum*. Moreover, *V.bubulus* was identified from the bovine vagina and semen whereas *V.fecalis* was isolated from normal ovine feces.

Due to their unusual growth characteristics such as microaerophilic and nonfermentative metabolism, the new genus *Campylobacter* was established consisting of *C.fetus, C.coli, C.jejuni*, and *C.sputorum*. Later in 1989, this genus had been an argument revised several times and finally the DNA-rRNA hybridization was used to classified this genus. Three rRNA homology clusters were found and classified into the genus *Campylobacter*, the genus *Arcobacter*, and the genus *Helicobacter*.

The family *Campylobacteraceae* consists of the genus *Campylobacter* and *Arcobacter*, which are phylogenic neighbors and share several genotypic and phenotypic features. This family is characterized by curved, s-shaped or spiral rod cells with 0.2 to 0.8 μm wide and 0.5-5 μm long. They are gram negative and nonsporeforming. Cells in old cultures may form spherical or coccid bodies. They are typically motile with a characteristic cock screw-like motion by means of a single polar unsheathed flagellum at one or both ends of the cells. Most of them are microaerophilic, nonfermentative with oxidase positive and variable in regard to catalase reaction . Nutritional basal medium supplement with 5-10 % of blood under reducing oxygen tension are the best way to culture. (Vandamme,2000)

## 2.2 *Campylobacter spp.* in animals

Many species of *Campylobacter* can be isolated from many species of animal (Table 1.1) (Quinn, et al.,1999). Many different animal species maintain *Campylobacter spp.* with no clinical signs (Steinhauserova, et al.,2001). The most important species of *Campylobacter* in veterinary medicine are *C. fetus* subsp. *fetus* and *venerealis*. Of *Campylobacter* spp. that is pathogenic in food animals, *C. fetus* can cause reproductive disorders in cattle and sheep (Quinn, et al.,1999), and *C. hyointestinalis* and *C. mucosalis* have been associated with enteritis in pigs and cattle (Garcia, et al.,1983).

*C.jejuni* and *C.coli* are the two most important species of concern in veterinary public health since humans commonly get infection from consumption of food of animal origin particularly chicken. The predominant species of *Campylobacter* in chicken is *C.jejuni* , which have the highest prevalence of isolation from chicken processing plant and retail raw chicken. The prevalence of *Campylobacter* contamination in chicken at poultry houses was 71.2% by the molecular and 65.7% by bacteriological techniques. The prevalence at the slaughterhouses was 34.7% and 44.8% by the same technique, respectively. However, the samples from supermarket showed the increasing prevalence of 75.71% from both techniques. *C.jejuni* was major species of *Campylobacter* at all levels of chicken production (Chattopadhyay, et al.,2001).

*C.coli* was the predominant species in pigs (32.9%), followed by *C.jejuni* (4.3%)(Ausubel, et al.,1997). *C.coli* was endemic in the plant and was

identified in fecal swabs and in all areas of the pork processing environment examined(Blaser, et al.,1986). *Campylobacter spp.* isolated from porcine rectal swabs, different areas in a pork slaughter and processing plant was *C.coli* (86.9%) and *C.jejuni* (13.1%). *Campylobacter* were also isolated from both dogs and cats. The predominant species was *C.jejuni* (52.9%), *C.coli*(17.6%), *C.upsaliensis* (25.5%) and *C.lari* (3.9%) (Michino and Otsuki,2000). *C.jejuni* was the most frequently isolated *Campylobacter spp.* from cattle (10%). *C. lari* was also identified in cattle (Ausubel, et al.,1997).

**2.3 *Campylobacter* infection in human**

*Campylobacter spp.* infection seems to pose several virulent factors. The production of bloody diarrhea, characterized by the presence of numerous polymorphonuclear neutrophils in patients with gastroenteritis and appearance of certain strains of *Campylobacter* in the blood of patients after ingestion of contaminated material, is indirect of the organism's invasive capacity.

*Campylobacter* (*C.coli, C.jejuni, C.lari*) are known to produce a cytotoxin, a cytotoxic factor, and an enterotoxin, which may account for those cases characterized by watery diarrhea. The actual importance of toxin production in development of disease is still being investigated. (Baron, et al.,1994)

*Campylobacter jejuni* is the important cause of human enteritis, which often found a transient watery diarrhea. The toxins play a role in this disease like other diarrheagenic bacteria pathogen, *Campylobacter* cytotoxin, cytolethal distending toxin (CDT) and non-CDT cytotoxins and enterotoxin.

For the infection to become established, *Campylobacter* must first survive the acidic condition in the stomach and then colonize the jejunum and ileum. Thus, lowering of gastric acidity facilitate infection, which is well established in relationship to *Salmonella* infection. Colonization of the gut mucosa depends on unimpaired bacterial motility and the ability to attach to the surface of mucosal cells. The rapid motility and spiral shape of *Campylobacter* enables them to move easily through viscous mucus overlying the mucosa.

The essential lesion is an acute inflammatory enteritis, which commonly extends down the intestine to affect the colon and rectum. Terminal ileitis and cecitis with mesenteric adenitis, are prominent features. Since autopsy or surgical material are rare, nearly all our knowledge of the histological changes in the gut are derived from biopsy specimens obtained at proctosigmoidoscopy.

The onset of *Campylobacter* enteritis is often abrupt, with cramping pains in the abdomen quickly followed by diarrhea, but about 30% of patients suffer a non specific influenza-like prodrome with one or more symptoms of fever, headache, dizziness, and myalgia. Rigors have been recovered in up to 22% of patients and fever may be sufficiently high to cause concusions in children or delirium in adults. The prodrome can be highly misleading in the absence of abdominal symptoms, which may not appear for 2 or 3 days. Patients with prodromal symptoms tend to have more severe illness than those whose illness starts with diarrhea.

The onset of diarrhea betrays the intestinal nature of the infection. It is commonly profuse, watery, bile stained, and sometimes protracting. At least 50% of patients attending emergency rooms have 10 or more bowel actions per day. After 1

or 2 days of diarrhea, frank blood appears in the stools in about 15% of patients, indicating a progression of infection into the colon and rectum.

Nausea is a frequent symptom, but only about 15% of patients vomit. A particular feature of *Campylobacter* enteritis is abdominal pain, which may become continuous and sufficiently intense to mimic acute appendicitis; this is the most frequent reason for admission of *Campylobacter* enteritis patients to the hospitals. After variable period, usually about 3 to 4 days into the illness the diarrhea begins to ease and the patient's condition improves, although the abdominal pain may persist for several days.

*C.jejuni* and *C.coli* associated with the most human infections are usually transmitted via contaminated food, milk, or water. Outbreaks have been associated with contaminated drinking water and consumption of improperly pasteurized milk, among other sources. In contrast to other agents of foodborne gastroenteritis including *Salmonella*, and *Staphylococci*, *Campylobacter* does not multiply in food.

In humans, *C.jejuni* and *C.coli* are the predominant species causing gastroenteritis. In developed countries, up to 99% of *Campylobacter* enteritis cases caused by *C.jejuni* (Friedman, et al.,2000). In Thailand, *C.jejuni* was the most frequently *Campylobacter* isolated from children with diarrhea (67%), followed by *C.coli* (15%) (On,1996). Many reports from various hospitals in Thailand also identified *C.jejuni* and *C.coli* in diarrhea children (Cloak and Reatamico,2002, Denis, et al.,2001, Fermer and Engvall,1999). *C.jejuni* IgA in healthy Thai villagers were also reported to progressively increased through life. However, IgG would reach highest level in the second year of life and IgM would reach the highest level during

late childhood and teenager period (Allos,2001). This observation suggested that Thai people were exposed to *Campylobacter* through their lives but have the highest risk of infection during childhood and teenage period.

**2.4 Risk factors of *Campylobacter* infection**

The risk factors for human Campylobacteriosis reported previously include consumption of chicken, meat, contact with animals particularly farm or zoo animals, attending a party and participating in water sport (Leksomboon, et al.,1981). Children age 0-35 months had increased odds of being infected with *Campylobacter* if the family owned a pet puppies (OR=16.58), petted chicken (OR=11.80) and consumed mayonnaise (OR = 4.13). However, these children were not found to be associated with the general risk factors such as consumption of untreated water, raw milk, undercooked chicken, untreated handling raw poultry (Taylor, et al.,1987).

The consumption of chicken was identified as an important risk factor. (Adegbola, et al.,1990, Ausubel, et al.,1997, Friedman, et al.,2000). However, the evidence from a prospective study did not show a temporal association between contamination of chickens and human campylobateriosis; particularly, during seasonal peaks, which suggested that many cases did not originate from chickens (Rautelin, et al.,1999). In Thailand, prevalence of *Campylobacter* in chickens was found to be 85% at the farm, 37% at the slaughterhouse and as high as 47% in chicken meat sold at fresh market (Helms, et al.,2003). The comparable prevalence of *Campylobacter* has been reported elsewhere in both chicken production 66.3% (Chattopadhyay, et al.,2001) and raw retail chicken 57% (Rautelin, et al.,1999).

**2.5 The situation of *Campylobacter* infection in Thailand**

Most patients had an abrupt onset of acute diarrhea with an average of 6 loose and watery stool per day. Disease generally improved within a week of hospital admission. Of the 4 pure *Campylobacter jejuni* cases one had 2nd degree malnutrition and measles and one had 3rd degree malnutrition (Poocharoen and Bruin,1986).

In Thailand, many studies have clarified the public health problems of *Campylobacter* infection. The early reports in Thailand were published during the 1981 to 1988, which showed the prevalence of diarrhea patients caused by *Campylobacter*. The 4% *C.jejuni* or *c.coli* were isolated from pediatric diarrhea from Children's hospital in Bangkok and 6.7% from diarrhea children patients in Chiang Mai province. However, this enteric pathogen did not indicate the causing of diarrhea in Thai children. (Leksomboon, et al.,1981, Rasrinaul, et al.,1988). Taylor (1986) reported the increasing incidence of *Campylobacter spp.* isolates 16% from 200 Thai children with mucoid or bloody diarrhea, while the study in 1987 among 100 infants in an orphanage in Bangkok presented 31% *C.jejuni* and 21% *C.coli*. (Taylor, et al.,1986) and (Taylor, et al.,1987). Of the 1,230 under 5 year-old children with diarrhea in 1988, *Campylobacter* was isolated 13.5%, which was one of the most frequently found bacteria in children; followed by *Enterotoxigenic E.coli(ETEC)* 13% and *Salmonella* 12% (Rasrinaul, et al.,1988). In 1991 the prevalence showed 15% from 631 Thai children with diarrhea, which were 10% *C.jejuni*, 2% *C.coli*, and 3% atypical *Campylobacter spp* by the membrane filter technique (Taylor, et al.,1991). A

case-control study of endemic diarrhea disease among 1,230 Thai children less than five years of age, *Campylobacter* was detected in 13%, of which 11.5% were *C.jejuni* and 1.7% *C.coli*. *C.jejuni* was the most significantly associated with diarrhea in children less than 12 month old (P=0.037), while *Salmonella* was associated with those less than three months of ages. *Campylobacter* infection usually occurred with watery diarrhea; only 14 were found with bloody diarrhea (Echeverria, et al.,1989). In a study published by Suwatano(1997), 14% of children under 5 year old with an acute diarrhea from children ward of King Mongkut Prachomklao hospital had *Campylobacter spp.*

Most *Campylobacter* cases were younger children and infection occurred mainly between the age of 1 and 2 (16% of all patients were in this group). One of the 3 patients in the 4-5 year group was positive. No *Campylobacter* was isolated from children older than 5 years (Poocharoen and Bruin,1986). A total of 416 children with an acute diarrhea illness, *Campylobacter* species were isolated from 18.8% of children <12 months of age, 12.3% of those 12-23 months, and 10.3% of those 24-59 months. The age-related decrease in isolation rates were statistically significant (p=0.04) (Taylor, et al.,1993).

Infection occurred sporadically throughout the year, no cases were encountered in the months of January, March, April, and August. There were no obvious peak-seasons but the 3 cases in October and November accounted for 21% and 25% ,respectively, of all admitted diarrhea cases in those months.(Poocharoen and Bruin,1986)

Of the 87% *Campylobacter* infection in children were infected with the same serotype and biotype found in food of animal origin. 64 *Campylobacter* strains

were isolated from 61 foods, *C.jejuni* was isolated from 46 and *C.coli* from 18. Of the 55 *Campylobacter* isolated from chicken intestine, 76% were *C.jejuni* and 24% were *C.coli*. Of the 7 *Campylobacter* isolated from pork 71% were *C.coli* and 29% were *C.jejuni*. Both *Campylobacter* strain isolated from beef were *C.jejuni*. *C.jejuni* serotypes 4, 28, and 36 and *C.coli* serotypes 8, 20, and 29, the most frequently isolated serotypes from food, were isolated from 57% (172/300) of children infected with *Campylobacter*. Two percent (1/46) of *C.jejuni* and 39% (7/18) of *C.coli* from foods were resistant to ≥ 8 μg/ml of erythromycin. Chicken was more often contaminated with *Campylobacter* than either pork or beef. While *C.jejuni* was the predominant biotype from chicken *C.coli* was predominantly in pork and was never isolated from beef. *C.coli* was significantly more often isolated from foods (28%) than from humans(16%, p=0.04). *Campylobacter* of human and food origin correlated by serotype. There were more nontypable strains among food isolates because there was a delay before serotyping was performed (Rasrinaul, et al.,1988).

Among orphanage-acquired strain, 53% of 43 *C.jejuni* strains and 91% of 23 *C.coli* strains were resistant to erythromycin compared with 11% of 114 *C.jejuni* and 46% of 35 *C.coli* strains that was community acquired. Erythromycin resistance is common among *Campylobacter* strains in Bangkok, especially in an institutional setting, which may account for the lack of efficacy of erythromycin for treatment of acute diarrhea illness (Taylor, et al.,1987).

Not only *Campylobacter* infection in children under 5 year old which was considered as a public health problem, but these pathogens were also identified from deployed U.S. military which had the diarrheal attack rate rage from 28%-40% within 1 month. *C.jejuni* was found to be the most common pathogen (25%) among

this U.S. troops, while the less frequently found were *E.coli*, nontyphoidal Salmonella, and Rotavirus (Echeverria, et al.,1993) and (BechamIII.H., et al.,1997). Besides, the studies among American Peace Corps volunteers during 5 weeks in rural Thailand did not indicate the important of *Campylobacter* infection as a causing of traveler's diarrhea (Echeverria, et al.,1981). Moreover, among the refugees in Thailand *Campylobacter* was also one of the most predominant pathogens where the highest diarrheal rate was in children under 1 year of age (Taylor, et al.,1988). In a study of chronic diarrhea in AIDS patients, *Campylobacter* was isolated 2.2% of the patients (Manatsathit, et al.,1996).

In Thai villagers, IgA began low and rose progressively throughout childhood and adolescence and continued to rise throughout later adult life. Whereas IgG levels fell quickly during the first few months of life and then rose steadily to peak during the second year of life at about three times the adult level. IgG levels subsequently fell until the second decade of life and thereafter remained relatively constant. IgM levels rose progressively until 2 years of age, remained elevated until 19 year old and then gradually declined during adulthood.(Taylor and Echeveria,1986)



**2.6 Laboratory diagnosis**

**2.6.1 Collection and transportation**

The demonstration of *C.jejuni* in a sample depends on the initial number of organisms, the sampling technique, and the efficacy of the culturing technique. Survival at a particular time depends on the inherent resistance of the

strains, the number of organisms initially inoculated onto a particular sample, and the size of sample examined. Many factors should be taken into consideration when preparing to transport *Campylobacters* as follow; 1) *Campylobacter* dies slowly at temperatures between 10 and 30°C, 2) The concentration of NaCl exceeding 2% and acidic environment are inhibitory, 3) They are sensitive to atmospheric of oxygen. Several transport media have been developed for fecal samples such as a semisolid brucella agar with 10% ovine blood without antibiotics, SIFF medium, Cary-Blair medium, which has been found to be the most efficient for preservation of *Campylobacter*. In conclusion, when fecal samples are collected for culture of *Campylobacter*, they should be sent to the laboratory without any delay and stored at 4°C. For stool samples that cannot be processed within 24 hour or for specimen with small numbers of *Campylobacter*, such as food, water, a transport medium should be used.

### 2.6.2 Isolation and identification of *Campylobacter spp.*

#### 2.6.2.1 Isolation of *Campylobacter* from blood culture

*C.fetus* subsp. *fetus, C.lari, C.jejuni* and *C.upsaliensis* have been documented as agents of sepsis and grow in most blood culture media, although they may require as long as 2 weeks for growth to be detected. Subcultures from broths must be incubated in a microaerobic atmosphere, or the organisms will not multiply. Turbidity is often not visible in blood culture media; therefore, blind subcultures or microscopic examination using acridine orange stain is necessary. The presence of *Campylobacter spp.* on blood culture is detected effectively by carbon dioxide ($CO_2$) monitoring. Isolation from sources other than blood or feces (extremely rare) is

ideally accomplished by inoculating the material (macerated tissue, wound exudates) to a non-selective blood or chocolate agar plate and incubating the plate at 37°C in a $CO_2$ enriched, microaerobic atmosphere. Selective agars containing cephalosporin, rifampin, and polymyxinB may inhibit growth of some strain and should not be used for isolation from normally sterile sites.

**2.6.2.2 Isolation of Campylobacter spp. from feces**

The most common agents of gastroenteritis, *C.jejuni* and *C.coli*, are able to grow well at 42°C and are resistant to cephalosporin, characteristic useful for their initial isolation. The number of colonies does not increase at this temperature, but the colonies appear sooner and are larger, and the growth of most normal fecal flora is inhibited. Feces may be transported to the laboratory directly for inoculation onto media. If a delay of longer than 2 hours is anticipated, material should be placed either in Cary-Blair transport medium or in Campy thio, a thioglycolate broth base with 0.16% agar and vancomycin (10 mg/L), trimethoprim (5mg/L), cephalothin (15mg/L), polymyxinB (2,500 U/L), and amphotericin B (2 mg/L). The same antimicrobial agents are incorporated into brucella agar base with 10% sheep blood to produce campy-BAP, one of the selective agars that is useful for cultivation of these strain. Several media manufacturers produce commercial plates for *Campylobacter* isolation. Since these *Campylobacter* are resistant to cold, refrigeration of fresh stools for as long as 24 hours before plating will probably not result in false- negative cultures. The stool should be plated onto a selective agar and incubated in 42°C in a microaerobic atmosphere. The atmosphere can be generated in several ways, including commercially produced gas-generating envelopes meant to

be used in conjunction with plastic bags or plastic jars. Evacuation and replacement in plastic bags or anaerobic jars with an atmosphere of 10% $CO_2$, 5%$O_2$, and the balance $N_2$ is the most cost-effective method, although it is somewhat labor intensive. Plates should be held for 72 hours before being discard as negative.

Filtration of feces is an excellent method for isolation of *Campylobacter spp*. Either a 0.65 or a 0.8 μm pore-size cellulose acetate filter can be used. The stool is diluted, if necessary, and several drops of filtrate are placed onto the agar surface, and a drop of stool is placed onto nonselective agar and incubated at 37°C in a microaerobic atmosphere. In a modification, a filter is placed onto the agar surface, and a drop of stool is placed on the filter. The plate is incubated upright. After 30 to 60 minutes at room temperature or 37°C, the filter is removed, and the plates are reincubated.

Plates should be examined at 48 hours and after 5 days for characteristic colonies, which are gray to pinkish or yellowish gray, slightly mucoid-looking colonies; some colonies may exhibit a tailing effect along the streak line. However, other colony morphologies are also frequently seen (Baron, et al.,1994).

### 2.6.3 Differentiation of *Campylobacter spp.*

Traditional technique for species identification of *Campylobacter* commonly utilize many differences in biochemical characteristics and susceptibility profiles to antimicrobial agents among different species(Vandamme,2000). Phenotypic testing is widely used in routine laboratories by biochemical tests for differentiating *Campylobacter*. This method has no standardization and lacks objectivity in the schema available (On,1996).

Hippurate hydrolysis has been used as the only phenotypic test to discriminate *C.jejuni* from *C. coli*. The hippurate gene only found in *C. jejuni* has been characterized and sequenced. But hippurate-negative isolates of *C. jejuni* occurred. Twenty eight thermophilic *Campylobacter* isolates showing negative hippurate hydrolysis were further characterized. Using *C. jejuni* and *C .coli* specific primers for *CeuE* gene, 5 isolates with repeatedly negative results in rapid hippurate hydrolysis were positive in *C.jejuni* specific PCR and 13 isolates were shown to be *C.coli*. In contrast to hippurate hydrolysis, PCR seemed to be a more reliable method to identify *C. coli* (Rautelin, et al.,1999).

Problems in species typing are most frequently encountered in the differentiation between *C. jejuni* and *C. coli*. The most important phenotypic discrimination test is the hippurate hydrolysis test, which is positive for *C. jejuni* only. The enzyme hippuric hydrolase catalyses the hydrolysis of hippuric acid to benzoic acid, a glycine, and the hippurate hydrolase gene is unique to *C .jejuni*. Another important problem in the typing of thermophilic *Campylobacter spp.* has been the incidence of significant amounts of nalidixic acid-resistant strains. Most of those strains, which carry a biochemical resemblance to *C. lari* strains themselves in pigs are rare. The mistake in phenotyping may be partly due to the variable ability of *C. coli* to produce hydrogen sulphide on TSI agar. Also, the 10-15% resistance of *C. upsaliensis* to cephalothin described in the literature may lead to errors in identifying those strains as *C. coli*. The present study also lists (Table2.1) porcine strains, biochemically identified as *C. upsaliensis*, that have been identified as *C. coli* by PCR and restrictive digestion.

Polymerase Chain Reaction (PCR) is a rapid procedure for in vitro enzymatic amplification of a specific segment of DNA (Ausubel, et al.,1997). By using DNA polymerase enzyme, a very small amount of DNA template can be amplified. Resulting product can be visualized by gel electrophoresis. The specificity of the assay depends on the primer, which can be designed to amplify a specific segment of DNA only. Therefore, DNA template without specific target for a specific primer will not yield any product.

One important application of PCR assay is to use it for identify species of bacteria which is difficult to differentiate using phenotypic methods such as *Campylobacter*. PCR assay for species differentiation of *Campylobacter* has been reported. In some instance, PCR can be coupled with other technique such as restriction fragment length polymorphism (RFLP) for *Campylobacter species* identification. Many PCR assay for *Campylobacter species* identification apply 12S rRNA gene amplification which has a conserved region common for all *Campylobacter* species and validity region which is different among *Campylobacter species*. This allows differentiation of *Campylobacter spp.* from other closely related bacteria and at the same time allows species differentiation among *Campylobacter*.

Most molecular method have marked an important use of 16S rRNA and 23S rRNA for identification of *Campylobacter spp.* However, other genes can also be used; *flaA* gene is used very frequently, based on PCR-RFLP digestion of sequence of the flegellin gene (Steinhauserova, et al.,2001). In addition, by two simple restriction enzyme digestions performed directly in the PCR mixture, it differentiates between species within this group of bacteria. Thus, the method has the potential to be used for the detection and identification of the thermophillic

*Campylobacter* in complex samples, such as food in which low numbers are present (Fermer and Engvall,1999). However, this technique require post PCR manipulation and gel electrophoresis has to be done twice.

A multiplex PCR assay is a PCR assay that uses several primers to competitively amplify target DNA templates. At least two multiplex PCR protocol for species identification of *Campylobacter* has been reported (Korolik, et al.,2001, Wang, et al.,2002). The protocol reported by Korolik *et al.* (2001) can only differentiate *C. jejuni* from other species of thermophilic *Campylobacter*. While the protocol reported by Wang et .al (2002) can be used to identify each of the five species of thermophilic *Campylobacter* in a single PCR reaction. The multiplex PCR assay proposed to use in this study (Wang, et al.,2002) employs primer specific to 23S rRNA gene common to all thermophillic *Campylobacter* species in order to differentiate thermophillic *Campylobacter* from other *Campylobacter*, and 5 other pairs of primers, each specific to different genes of different species of *Campylobacter*. This would allow species differentiation among thermophillic *Campylobacter*. This assay can be carried out in single PCR reaction which saves time and reagents. Results of specific identification can be determined by observing products specific to each species of *Campylobacter* by gel electrophoresis.



**2.7 Antibiotic resistance of *Campylobacter spp.***

Emergence of antimicrobial resistance has become a serious problem worldwide. While much of the resistance observed in human medicine is attributed to inappropriate use of antibiotics in humans, there is increasing evidence that

antimicrobial use in animals selected for resistance foodborne pathogens that may transmitted to human as food contamination (White, et al.,2002).

Most antibiotics used therapeutically were imported for human than for veterinary use, but agents such as chlortetracycline, oxytetracycline, sulphonamides, lincomycin or tylosin (which may be used therapeutically in people or belong to the same family as human therapeutic antimicrobial agents) are imported in substantially greater quantities for veterinary or growth promotant use.

Antibiotic treatment leads to the expression of resistance in exposed bacterial populations. Antibiotic resistant bacteria are more frequently isolated from pigs and poultry than from extensively grazed cattle or sheep, reflecting the greater use if antimicrobial agents in intensively housed species. It is clear that bacteria-carrying genes that control the expression of antibiotics resistance can pass between animals and people and the environment. Growth promotant improve growth rate and feed utilization and lead to saving in land use for feed production.

Feeding at small dose rates of antibiotics (as with growth promotant compounds) was recognized more than 20 years ago to increase the antibiotic resistance of gut flora. Treatment of chickens with small concentrations of ampicillin has been shown to lead to the dependent of high-level (MIC > 100 μg/ml) resistance to *E.coli* in chickens. There is also evidence that long-term exposure of animals to antimicrobial agents may prolong fecal shedding of pathogen such as thermophilic *Campylobacters, Salmonella*, and *Listeria*.

Fluoroquinolone-resistant thermophilic *Campylobacter spp*. is concern as a usually human intestinal infection. *Campylobacter spp* are self-limiting, but

there are occasions when treatment is warranted and the high in-vitro activity of fluoroquinolones against these organisms indicates their potential for clinical use.

Fluoroquinolone are not registered for use in farm animals in Australia and to date fluoroquinolone resistance in humans. *Campylobacters* or *Salmonellas* has not been recorded. A small study in South Australia has found no fluoroquinolone resistance in thermophilic *Campylobacters* isolated from pigs (Barton,1998)

Of the 68 Campylobacter isolates from broilers in southern part of Japan (32.4%) were determined quinolone-resistant *Campylobacter*. All the isolates except one were cross-resistant to nalidixic acid, ofloxacin, and norfloxacin. A high frequency if quinolone resistance was found in both *C.jejuni* and *C.coli*, whereas a high level of Erythromycin resistance was found only in *C.coli*. All *C.jejuni* isolates were sensitive to erythromycin.(Chuma, et al.,2001)

*C.jejuni* and *C.coli* are generally susceptible to a variety of antimicrobial agents; however, increasing resistance to some drugs has been documented. A Canadian study (Gaudreau and Gilbert,1998) showed that resistance to tetracycline increased in *C.jejuni* isolates from 19.1% to 55.7% between 1985-1995. More than 12% of 1995-1997 isolates were resistant to quinolones (nalidixic acid and ciprofloxacin), whereas all of isolates from 1985-1986 were susceptible to these agents. Among the *C.jejuni* isolates of human origin tested through the US National Antimicrobial Resistance Monitoring System (NARMS) in 1999, 54% were resistance to one or more antimicrobial agents. The most common resistance phenotypes observed were to tetracycline (46%), nalidixic acid(20%) and ciprofloxacin(18%). Among the *C.coli* isolates, 50% were resistant to one or more

antimicrobials, and 35% was resistant to two or more agents. The most common resistance among the *C.coli* isolates was nalidixic acid (30%), followed by tetracycline and ciprofloxacin (30% each).

In the US, there are no reports of ciprofloxacin-resistant human *Campylobacter* isolated prior to 1992. The incidence of fluoroquinolone resistance among NARMS isolates in 1999 was 18%, up from 13% in 1997. This increase coincided with the licensing of fluoroquinolones for treating colibacillosis in poultry, leading to concerns that association was causal. A similar association was seen in the Netherlands, where the emergence of fluoroquinolone-resistant human *C.jejuni* infection followed the advent of poultry use in 1987 (White, et al.,2002). In Minnesota, the annual percent-age of quinolone-resistant infections increased from 1.3% in 1992 to 10.25 in 1998. In 1997, 2 years after the licensing of safrofloxacin in 1995, resistance among *Campylobacter* in Minnesota doubled . Although foreign travel accounted for some of this increase, acquisition of resistant strains from poultry was identified as an important factor (Smith, et al.,1999). In other countries where antimicrobials are less restricted, fluoroquinolone-resistant *Campylobacter* is a bigger problem. In a report from Spain, 88% of *Campylobacter* isolates displayed fluoroquinolone resistance. (Ruiz, et al.,1998)

**Table 2.1** Pathogenic and nonpathogenic *Campylobacter species*

| Species | Principal host (s) | Disease and/or commensal status |
|---|---|---|
| *C.fetus subsp.venerealis* | Cattle | Bovine genital campylobacteriosis (epizootic bovine infertility): Infertility, early embryonic death and occasional abortion. Prepuce of symptomatic bulls |
| *C.fetus subsp. fetus* | Sheep | Ovine genital campylobacteriosis:outbreaks of abortion |
| | Cattle | Occasional abortion |
| | Man | Occasional infection |
| | Cattle, sheep | Commensal in the intestinal tract |
| *C.jejuni* | Sheep | Outbreaks of abortion |
| | Dogs, cat, other animals and man | Enteritis with diarrhea |
| | Poultry, cattle | Avian vibrionic hepatitis |
| | Many domestic and wild animals and birds | Commensal in the intestinal tract |
| *C.mucosalis* | Pigs | Associated with the porcine intestinal adenomatosis complex. Present in the intestinal of normal pigs |

**Table 2.1** Pathogenic and nonpathogenic *Campylobacter species* (continued)

| Species | Principal host (s) | Disease and/or commensal status |
|---|---|---|
| *C.coli* | Pigs and man | May cause mild diarrhea in pigs and enteritis in man. Commensal in the intestinal of pigs. Tends to increase in numbers in pigs with swine dysentery caused by *Serpulina hyodysenteriae*. |
| *C.cryaerophila* | Cattle, pigs, sheep, horse | Isolated from faeces of normal animals and infrequently from aborted fetuses. Significance unknown |
| *C.laridis* | Dogs, horse and birds | Isolated from faeces. Disease status uncertain |
| *C.sputorum biovar sputorum* | Man | Commensal in oral cavity. Considered nonpathogenic |
| *C.spuotrum biovar bubulus* | Cattle | Cammensal in preputial cavity of bulls and genital tract of cows. Considered to be nonpathogenic |
| *C.sputorum biovar fecalis* | Sheep, cattle | Present in the intestinal tract and has been isolated from semen and vagina of cattle. Considered to be nonpathogenic |
| *C.upsaliensis* | Dogs, man | Isolated from diarrhea and normal individuals. Disease status uncertain. |

**Table 2.2** Differential characteristics of clinically relevant *Campylobacter spp*

| Genus/ Species | Growth at | | Hippurate hydrolysis | Catalase | $H_2S$ in Triple sugar iron agar | Indoxyl Acetate Hydrolysis | Nitrate To Nitrite | Susceptible to 30 μg disk | |
|---|---|---|---|---|---|---|---|---|---|
| | 25°C | 42°C | | | | | | cephalothin | nalidixic acid |
| *C.coli* | - | + | - | + | - | + | + | - | + |
| *C.concisus* | - | + | - | - | + | - | + | - | - |
| *C.curvus** | - | + | - | - | + | + | + | ND | + |
| *C.fetus* Subsp. *fetus* | + | -/+ | - | + | - | - | + | + | - |
| *C.hyointestinalis* | +/- | + | - | + | + | - | + | + | - |
| *C.jejuni* | - | + | + | + | - | + | + | - | + |
| *C.jejuni* Subsp.*doylei* | - | +/- | + | +/- or weak+ | - | + | - | + | + |
| *C.lari* | - | + | - | + | - | - | + | - | - |
| *C.rectus** | - | slight + | - | - | + | + | + | ND | + |
| *C.sputorum* | - | + | - | -/+ | + | - | + | + | -/+ |
| *C.upsaliensis* | - | + | - | -/weak+ | - | + | + | + | + |

+, most strain positive; -, most strain negative;+/-, variable(more often positive);-/+, variable(more often negative);ND,test not done

*Anaerobic, not microaerobic