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Review

Detection of *Campylobacter jejuni* from food and its epidemiology

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Food borne campylobacteriosis is distributed all over the world. Large outbreaks have been associated with consuming raw or inadequately pasteurized milk and contaminated water. *Campylobacter jejuni* is not an environmental organism rather zoonotic organism. It habituates intestinal tract of a wide range of warm blooded animals. The principal route by which *C. jejuni* contaminates the food is through fecal contamination by *C. jejuni* infected carriers. Raw meats and poultry become contaminated during processing when intestinal contents contact the meat surfaces. *C. jejuni* is one of the most common causes of bacterial diarrhoeal disease worldwide. As an alternative to growth on agar, there are a variety of technologies which may provide rapid diagnostic results such as immunoassay methods; molecular methods such as polymerase chain reaction (PCR)/nucleic acid techniques which reduce the time to result such as concentration using cell separation. *Campylobacter* impedes the public health problem and incurs severe economic losses in industries processing food of animal origin. Reinforcing hygienic practices at each link in the food chain from producer to consumers is critical in preventing the disease.

Key words: *Campylobacter jejuni*, food, poultry, public health.

INTRODUCTION

Awareness of the public health implications of Campylobacter infections has evolved over more than a century. In 1886, Escherich observed organisms resembling campylobacters in stool samples of children with diarrhea. In 1913, McFaydean and Stockman identified campylobacters called related Vibrio in fetal tissues of aborted sheep. In 1957, King described the isolation of related Vibrio from blood samples of children with diarrhea, and in 1972, clinical microbiologists in Belgium first isolated campylobacters from stool samples of patients with diarrhea. The development of selective growth media in the 1970s permitted more laboratories to test stool specimens for *Campylobacter*. Soon, *Campylobacter* species were established as common human pathogens. *Campylobacter jejuni* infections are now the leading cause of bacterial gastroenteritis reported in the United States (Sean et al., 1999).

C. jejuni can colonize the intestinal tract of most mammals and birds and are the most frequently isolated *Campylobacter* species in humans with gastro-enteritis. Transmission from animals to humans is mainly through consumption and handling of contaminated animal food

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products particularly poultry and improperly treated water but also direct contact with carrier animals may contribute to human campylobacteriosis (Sean et al., 1999).

C. jejuni does not cause clinical disease in adult animals except for sporadic cases of abortion in ruminants and very rare cases of hepatitis in ostriches. In humans, extra intestinal infections, including bacteraemia, can occur and some sequelae of infection, such as polyneuropathies, though rare, can be serious (Stern, 1992). The faecal contamination of meat, especially poultry meat, during processing is considered to be a major source of human food-borne disease. Hence the objective of this paper is to assess the detection methods of *C. jejuni* from food and its epidemiology.

GLOBAL OCCURRENCE

Food borne campylobacteriosis is distributed all over the world. Large outbreaks have been associated with consuming raw meat or inadequately pasteurized milk and contaminated water. The greatest incidence of *C. jejuni* infection is seen in infants, young adults and immune compromised groups. There is also a seasonal trend in incidence that is highest in spring/early summer (mid-June and mid-July) due to informal eating outside, such as barbecues, coupled with an increase in temperature and agricultural activities (Mandrell et al., 2006). Epidemiological studies showed that cross contaminations during defeathering/skinning and evisceration play important roles in the occurrence of campylobacteriosis in poultry (Pamuk and Akgun, 2009). Wild birds are considered to be an important reservoir of infection for domestic and food animals as well as poultry which is a natural host for *Campylobacter*. *C. jejuni* is not an environmental organism rather zoonotic organism. It habituates intestinal tract of a wide range of warm blooded animals like birds, cattle, sheep, pigs, goats and domestic pets, (especially puppies and kittens) (Doyle and Beuchat, 2007). *Campylobacter* species can also colonize the reproductive organs and oral cavities of animals and humans.

Etiology

C. jejuni is Gram negative, oxidase and catalase positive, non spore forming, spiral in shape, with corkscrew like darting motility. It possesses a single polar flagellum and belongs to the family Campylobacteriaceae (Rollins and Joseph, 2001). The genus *Campylobacter* consists of 16 species. The commonest human pathogens are *C. jejuni, Campylobacter coli* and *Campylobacter lari.* The most significant pathogens, C. *jejuni* and C. *coli* can only grow at temperatures above 30°C and are called thermophilic with optimum growth temperature of 42 to 43°C. *Campylobacter* is microaerophilic and will grow best at an atmosphere of 10% carbon dioxide, 56% oxygen and some amount of hydrogen (Doyle and Beuchat, 2007).

Transmission

The principal route by which *Campylobacter* contaminates the food is through fecal contamination by *Campylobacter* infected carriers. Raw meats and poultry become contaminated during processing when intestinal contents contact the meat surfaces. Mostly human campylobacteriosis are associated with handling of raw poultry, undercooked contaminated meat, cross contamination of raw and cooked foods and poor hygiene (Suzuki and Yamamoto, 2009). Feco-oral person to person transmission of infection has been reported for *C. jejuni.* This uncommon type of transmission can occur when personal hygiene is poor. Humans act as vectors transferring the organism into poultry production area with contaminated clothing and foot wear (Doyle and Beuchat, 2007).

Pathogenesis

The pathogenesis of Campylobacteriosis is not fully understood. The diarrheal disease may be due to the production of a heat-liable toxin. The flagellum which has a coded flagellin gene (flaA) enables the bacterium to reach the attachment sites in the intestine. The pathogenesis involves host and pathogen specific factors, the health and age of the host and pathogen specific humoral immunity from previous exposure which influence clinical outcome after infection (Konkel et al., 2004). Several putative virulence factors have been identified in *Campylobacter* which contribute to the motility, intestinal adhesion, colonization, toxin production and invasion. Adhesion of the pathogen to the intestinal epithelium is important for colonization and to increase the secretion of bacterial toxins (Thakur et al., 2010).

Clinical signs

The illness caused by *C. jejuni* is not easily distinguished from other types of gastro intestinal disease (GIT). Clinical signs vary widely from mild to quite severe illness and usually last for 1 to 7 days, but sometimes for several weeks. Abdominal pain can persist for up to 7 days and recurrence of symptoms can occur. The illness may start with cramping abdomen, diarrhea, fever, chills, headache, myalgia and occasionally delirium, with typical

more intense long lasting abdominal pain and occasionally blood or mucous in the stool (Doyle and Beuchat, 2007).

Treatment

The majority of *Campylobacter* infections are sporadic and self-limiting which makes difficulty to accurately determine the true incidence rate; due to this, antimicrobials are usually not recommended except in severe cases (Yates 2005). For treating *Campylobacter* infections, fluoroquinolones (e.g., ciprofloxacin) and macrolides (e.g., erythromycin) are the drugs of choice; however, treatment with erthryomycin does reduce the length of excretion. The frequency of resistance to these important classes of antimicrobials has reported to be on the rise in the United States and globally (Rozynek et al., 2008).

PUBLIC HEALTH SIGNIFICANCE

Thermophilic *Campylobacter* species have received considerable attention in recent years as a major cause of bacterial enteritis in man. *Campylobacter* enteritis is recognized as an important source of diarrheal illness worldwide. The pathogen is also an important causative agent of 'traveler diarrhea' accompanied by predisposing debilitating factors such as pregnancy, premature birth, chronic alcoholism, neoplasia and cardiovascular disease (Mandrell et al., 2006). Campylobacteriosis affects all age groups; however, infections are recognized with increasing frequencies in infants, children, aged individuals, and immune-compromised persons. According to the Centre for Disease Control (CDC) report, *Campylobacter* infections accounted for approximately one-third of laboratory confirmed food borne illness that occurred globally in food net surveillance areas (CDC, 2008). A serious consequence of diarrheal diseases in human is called Guillain-Barrè syndrome (GBS) which is characterized by polyneuritis of the peripheral nerves that may lead to either short term or lengthy paralysis. GBS, a demyelating disorder resulting in acute neuromuscular paralysis, is serious sequelae of *Campylobacter* infection (Shane, 2000).

ECONOMIC SIGNIFICANCE

Campylobacteriosis cause severe economic loses both in the public health and food industry sector. Campylobacteriosis has an enormous economic impact in terms of treatment costs, lost of production, and human welfare. In livestock, particularly sheep and cattle, *Campylobacter* species are the cause of important economic losses associated with infertility problems and abortion (Beatriz and Ana, 2011).

Control and prevention

C. jejuni grows easily if contaminated foods are left out at room temperature; however, the bacterium is sensitive to heat and sterilization methods like pasteurization of milk, cooking meat, and water chlorination. To prevent *Campylobacter* infection, make sure that any poultry products are cooked at 74°C and choose the coolest part of the car for transportation of meat and poultry as well as defrost meat and poultry in the refrigerator and never leave food at room temperature for over two hours, wash hands after contact with pets or farm animals (Doyle and Beuchat, 2007).

Detection of *C. jejuni* from food

C. jejuni is one of the most common causes of bacterial diarrhoeal disease worldwide. This significant zoonotic pathogen is reported to have a low infective dose with high pathogenicity. Poultry and poultry products have long been associated with campylobacter infection, though a variety of food materials and other vectors have been implicated in the transmission such as unpasteurized milk, and water (Bang et al., 2001). Evaluation of food samples for the presence of *Campylobacter* can be challenging. Isolation of the organism from highly-contaminated samples may require different media depending on the food type and with incubation under microaerobic conditions. They are not the fastest growing organisms in which it can take up to a week to obtain a final test result (Bang et al., 2001).

DETECTION TECHNIQUES

In food and feedstuffs, sample is added to selective enrichment broths which can be obtained as base powder to which supplements may be added or ready-to-use formats. These are incubated at 37°C for 4 h and then at 41.5°C for 44 h. Selective agars either in powder format or ready-to-use are inoculated from this enrichment and incubated for a further 48 h. Clinical samples are sub-cultured directly to selective agars. Incubation atmosphere is critical for recovery of *Campylobacter*, microaerophilic conditions must be provided. Several proprietary atmosphere systems are available for this purpose. During the broth enrichment, a 10 to 15% aerobic headspace is sufficient (Martin et al., 2002). As an alternative to growth on agar, there are a

variety of technologies which may provide rapid results such as antibody/antigen interactions using immunoassay methods; molecular methods such as PCR/nucleic acid techniques which reduce the time to result such as concentration using cell separation. Sometimes combinations of these techniques are used to further enhance the speed to result. PCR can eliminate the need for identification in the event of a positive result and may also provide quantitative information. Quality control organisms are available to ensure that method performance is within standard criteria (Martin et al., 2002).

Sample preparation and processing

Samples are collected using sterile instruments, under aseptic condition. 25 g sample of food is put into a sterile stomacher bag, mixed with Bolton broth nine times the weight or volume. This is homogenized for 2 min to get homogenized sample (NSM, 2007).

Surface rinse technique

This is done by rinsing the surface of the sample then shaking or massaging it with 250 ml of nutrient broth (without agar) in a sterile plastic bag and filtering through two layers of cheese cloth and centrifuging the filtrate at 16,000 rps for 20 min. Finally, supernatant fluid is discarded and the pellet is suspended in a minimum (2 to 5 ml) cubic volume of pre-enrichment broth (NSM, 2007).

Swab technique

This is done by dipping a sterile swab into an enrichment broth and pressing the swab against the container wall to remove excess moisture. The carcass is then swabbed with the moist swab and the swab is put in pre-enrichment broth to incubate at 37°C for 4 to 6 h. There is no statistically significant difference in the isolation rates of *Campylobacter* species on carcass in different swabbing sites (Woldemariam et al., 2009).

Recognition of colonies

The plates should be examined as quickly as possible after removal from microaerobic environment for characterization. *C. jejuni* has gray/moist flat, glossy, effuse colony with a tendency to spread along the inoculation track having well spaced colonies resembling droplets of fluid and on moist agar a thin, spreading film and with continued incubation colonies become convex often with a dull surface (NSM, 2007).

Confirmatory tests

For oxidase test, immerse a swab in freshly prepared oxidase reagent and touch lightly the surface of the colony to be tested, the immediate appearance (in 10 s) of a dark purple color at the point of contact denotes a positive reaction which confirms *C. jejuni*. For microaerobic growth test, subculture suspected colonies from *Campylobacter* selective agar into two blood agar plates, then incubate one plate in microaerobic condition and the other aerobically at 41.5 ± 1°C to 22 ± 1 h. The growth in micro-aerobically incubated plates and no growth in aerobic conditions in line with other tests confirm the test (Chaban et al., 2010). As optional, cell morphology and motility tests can also be used by preparing a wet preparation and using phase contrast microscope. If *Campylobacter* species are present, there will be highly motile, slender rods with curved morphology and a characteristic darting or corkscrew like movement. Agglutination under normal lighting conditions indicates that the test organism is *C. jejuni* (Chaban et al., 2010).

CONCLUSION

Campylobacter species are the common bacterial pathogens causing gastroenteritis in both human and animals throughout the world. Raw meat from food animals could serve as potential source of *C. jejuni* indicating possible risks of infection to people. Consumption of poultry meat is suspected to be the leading causes of illness followed by ruminants' meat, unpasteurized milk, contaminated water and animal contact. In slaughter houses, contamination of carcasses occurs during dressing, skinning, evisceration and further meat processing steps. *Campylobacter* infection impedes the public health problem and incurs severe economic losses in industries processing food of animal origin. Reinforcing hygienic practices at each link in the food chain from producer to consumers is critical in preventing the disease.

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