Diagnosis of Campylobacter Infection In Cases With Diarrhea

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Summary and conclusion

Campylobacter (curved rod in Greek) may have been first discovered in the late nineteenth century (1886) by Theodor Escheric who observed it in the colon of infants who died of a disease, he named "cholera infantum" **(Bhunia, 2008).** Currently, of the 19 Campylobacter species/subspecies that have been classified, 10 or 11 have well-established associations with animal or human diseases **(Gilbreath et al., 2011).**

Campylobacter is a major food borne pathogen of animal origin, prominently associated with poultry and is responsible for a significant percentage of intestinal infectious diseases in human in the developed world and in developing countries. It is associated mainly with illness in children. In industrialized countries, sporadic cases of human infection are generally attributed to the consumption of raw or undercooked poultry meat whereas outbreaks have been traced to contamination of the water supply and the consumption of contaminated raw milk **(Peterson, 2003 and Jeon et al., 2010).**

The incidence of human campylobacteriosis increased exponentially during the last decade of the 20th century, although part of this increase can be attributed to better

detection of Campylobacter and better diagnosis **(Smith and Bayles, 2006).**

Campylobacteriosis is a self-limited disease and antimicrobial therapy is not generally indicated. However, treatment can reduce the duration and the severity of illness if it is initiated early in the course of infection **(Coker et al., 2002 and Adekunle et al., 2009).**

Campylobacter infection represents a significant and persistent public health problem, with approximately 10 % of cases requiring hospital treatment. Sequelae that may accompany illness, such as reactive arthritis, toxic megacolon and autoimmune-mediated demyelinating neuropathies (e.g Guillain-Barré and Miller Fisher syndromes) have been added to the disease burden **(Gillespie et al., 2006).**

Because poultry, livestock, pets, and wild animals are the major reservoir for Campylobacter, control is based on interruption of transmission to humans from animals, animal products, or environmental sources contaminated by animals **(Humprey and Jørgensen, 2006).**

The identification of Campylobacter using conventional methods is slow (culture-based methods can take up to five days) and problematic due to their fastidious growth requirements and biochemical inertness. Moreover, the detection of C. coli and/or C. jejuni in complex substrates like

faeces or environmental samples is difficult as the culture conditions have to be selective enough to avoid overgrowth from competiting organisms. PCR has provided a reliable tool to detect and to quantify C. jejuni and/or C. coli in pure culture, in poultry, milk, or water, and in complex substrates like food products and faecal samples **(Maridor et al., 2011).**

A number of simple and rapid identification and discrimination methods based on the PCR have been developed for thermophilic campylobacters on the basis of genetic diversity. Alternatively, multiplex PCR for targeting several species-specific genes have also been developed **(Persson and Olsen, 2005).**

Accurate identification of Campylobacter species, particularly C. jeuni, C. coli and C. fetus, provides important data for diagnosis and proper treatment, drug of choice for severe Campylobacter infection, epidemiologic surveillance and risk assessment studies **(Asakura et al., 2008).**

In the current study, 50 stool samples were collected from patients suffering from diarrhea with fever, headache, abdominal pain, myalgia, vomiting and/or blood in stool.The samples were subjected to Physical examination, microscopic examination, and culture on Skirrow's media with phenotyping by Hippurate test in addition to genotyping by PCR technique.

In the present study, Campylobacter was isolated on Skirrow's media from 6/50 (12%) stool samples obtained from patients with sensitivity, specificity, PPV and NPV of 100%. The 4/6 samples were identified as Campylobacter jejuni (Hippurate test +ve). The PCR confirmed the 6 positive samples as Campylobacter. Also, PCR identified the 4/6 Hippurate +ve test as C. jejuni and 2/6 Hippurate –ve test were identified as C. coli with sensitivity, specificity, PPV and NPV of 100%.

Our results revealed a highly significant association between the results of the Skirrow's media and PCR ($P<0.001$); also between all the risk factors and positive stool culture cases for Campylobacter (2 C. jejuni associated with type of food and 1 C. coli associated with contact with birds). A highly significant association between Campylobacter positive cases with fever (4/5 cases were due to C. jejuni) was found. Stool WBC $\geq$ 50 was significantly associated with stool culture positive cases

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List of Abbreviations

AFLP..... Amplified fragment length polymorphism

AIDP ..... Acute inflammatory demyelinating polyneuropathy

AIDS..... Acquired Immune Deficiency Syndrome

AMAN..... Acute motor axonal neuropathy

AMSAN..... Acute motor-sensory axonal neuropathy

ATP……...Adenosine triphosphate

BAPs..... Blood agar plates

C. ……. ..... Campylobacter

CA ..... Campylobacter agar

CAMPs..... Cationic antimicrobial peptides

cAMP……..….cyclic adenosine monophosphate

Campylobacter spp. Campylobacter species

CAT medium Cefoperazone , Amphotericin B, and Teicoplanin medium

CCDA..... Charcoal cefoperazone deoxycholate agar medium

CDC..... Center for Disease Control and Prevention

CDT..... Cytolethal distending toxin

CFU..... Colony forming unit

Cia ..... Campylobacter invasion antigens

CLSI..... Clinical and Laboratory Standards Institute

CPS..... Capsular polysaccharide

CSM ..... Charcoal-based selective medium

ECM..... Extracellular matrix

Fn ..... Fibronectin

GBS..... Guillain–Barré Syndrome

HIV..... Human Immunodeficiency Virus

HL antigens..... Heat labile antigens

HSP ..... Heat-shock proteins

IAH..... Indoxyl acetate hydrolysis

LOS ..... Lipooligosaccharide

LPS..... Lipopolysaccharide

MAMA ..... Mismatch amplification mutation assay

mCCDA..... Modified charcoal cefoperazone deoxycholate agar

MIC ..... Minimum inhibitory concentration

MLS_B ..... Macrolide lincosamide and streptogramin B

MLST ..... Multilocus sequence typing

MSCRAMMs .. Microbial surface components recognizing adhesive matrix molecules

PCR..... Polymerase chain reaction

PFGE..... Pulsed field gel electrophoresis

pgl..... Protein glycans

PMN ..... Polymorphonuclear leukocytes

QRDR..... Quinolone resistance-determining region

RFLP ..... Restriction fragment length polymorphism

T3SS ..... Type III secretion system

TMAO..... Trimethylamine oxide

TSI..... Triple sugar iron agar

US..... United States

V fetus ..... Vibrio fetus

INTRODUCTION

Campylobacter is widely acknowledged as one of the most frequent pathogens causing acute bacterial gastroenteritis worldwide. Campylobacter jejuni and Campylobacter coli are the predominant cause of Campylobacteriosis. Furthermore Campylobacter fetus, Campylobacter lari and Campylobacter upsaliensis have also been recognized as human pathogens causing gastroenteritis and/or septicemia ***(Matsune et al., 2007).***

Campylobacter jejuni (C. jejuni) causes an acute diarrheal disease with a variety of clinical symptoms, such as fever, diarrhea, headache, abdominal pain, myalgia, vomiting, and blood in stool ***(Haddad et al., 2009).***

Campylobacter infection represents a significant and persistent public health problem, with approximately 10 % of cases requiring hospital treatment. Sequelae that may accompany illness, such as reactive arthritis, toxic megacolon and autoimmune-mediated demyelinating neuropathies (e.g Guillain-Barré and Miller Fisher syndromes) have been added to the disease burden ***(Gillespie et al., 2006).*** This has emphasized the need for more rapid and efficient detection methodologies than the slow and complicated process of detecting Campylobacter by culture-based techniques ***(Olsen et al., 2009).***

Conventional diagnostic methods utilizing a combination of culture and biochemical testing require that suspected stool specimens are cultured on selective agar at 42°C under microaerophilic conditions for up to 72 hours before a negative report is issued ***(Al Amri et al., 2007).***

A number of phenotypic and molecular typing methods are used to identify outbreak–associated Campylobacter strains in specific food-borne or waterborne outbreaks ***(Forbes et al., 2009).*** The ability to distinguish between Campylobacter species is important in the identification of Campylobacter sources and transmission routes. The PCR assays that distinguish between one or more combinations of the thermotolerant Campylobacter species have been described ***(Klena et al., 2004)***.

Erythromycin was the first macrolide to treat Campylobacter infections and it remains the treatment of choice for patients with uncomplicated enteritis in many countries. Also tetracycline was used for many years for treatment of humans infected with C.jejuni and C.col ***(Gu et al., 2009).***

The resistance of C. jejuni to a range of antibiotics is common throughout the world and is thought to have been driven by the frequent use of antibiotics in animals farmed for meat. The genetic basis for antimicrobial drug resistance is known, and its spread by recombination has been demonstrated both within C. jejuni and between related species ***(Wilson et al., 2009).***