Bio Surveillance of *Campylobacteriosis* as Food Borne Illness in Egypt by Recent Accurate Diagnostic Methods

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Abstract: Campylobacteriosis is a common zoonotic disease that affect human and cause gastrointestinal disturbances'. Poultry meat is the primary source of human infection. Non-pasteurized milk, milk products, meat, meat products and fish's considered the important sources of transmission of the disease to human. ***Campylobacter jejuni*** (***C. jejuni***)is one of the most common cause's inflammations of the stomach and intestine of human's. *Campylobacter* is well recognized as the leading cause of bacterial foodborne diarrheal disease worldwide. Symptoms can range from mild to serious infections of the children and the elderly and permanent neurological symptoms. The organism is a cytochrome oxidase positive, microaerophilic, curved Gram-negative rod exhibiting corkscrew motility and is carried in the intestine of many wild and domestic animals, particularly avian species including poultry. Intestinal colonization results in healthy animals as carriers. This is review aims to discussing the (i) genus *Campylobacter* characteristics; (ii) detection and isolation of *Campylobacter*; (iii) campylobacteriosis and presence of virulence factors; and (iv) control strategies.
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Introduction:

(i) Genus *Campylobacter* characteristics

One effect of campylobacteriosis is tissue injury in the gut. ***C. jejuni*** is a bacterium commonly found in the guts of birds and mammals. The sites of tissue injury include the jejunum, the ileum, and the colon. C jejuni appears to achieve this by invading and destroying epithelial cells **(World Health Organization, 2007).**

It is believed that the first report concerning *Campylobacter* was back in1886 by The odore Escherich who observed and described non- culture able spiral-shaped bacteria **(Vandamme,2000; King and Adams,2008; Vandamme *etal*.,2010).**

Doyle isolated a different vibrio from feces of pigs with diarrhea and classified them as *Vibrio coli* **(Vandamme, 2000; Vandamme *etal*., 2010).**

Campylobacter by noting their high incidence in human diarrhea **(On, 2001).** Other author shave stated that there are16species with a further six subspecies within the genus *Campylobacter* (**On, 2001; Foster etal.,2004).** Campylobacters have been known to be the cause of diseases in animals since1909,but they have been generally recognized as a cause of human disease, only since about1980. The family Campylobacteraceae consists of two genera, *Campy- lobacter* and *Arcobacter* and occur primarily as commensals in humans and domestic animals **(Vandamme, 2000).**

Campy- lobacter jejuni hydrolyzes hippurate, in doxylacetate and reduces nitrate. Most strains are resistant to cephalothin, and also resistance to fluoroquinolones, a category of antibiotics normally used to treat animal and human illness, has been reported **(Koenraad et al., 1995).**

Thermo philic *Campylobacter* species are able to grow between 37 and 42˚C, but in capable of growth below 30˚C(absence of colds hock protein genes which play a role in low-temperature adaptation), with an optimum temperature of 41.5˚C. **Levin (2007)** suggested that these organisms should be referred to as "thermotolerant" since they do not exhibit true thermo phily (growth at 55˚C or above). Freezing–thawing also reduces the population of *Campylobac- ter* spp. **(Stern and Kazmi, 1989).**

Occurrence and severity

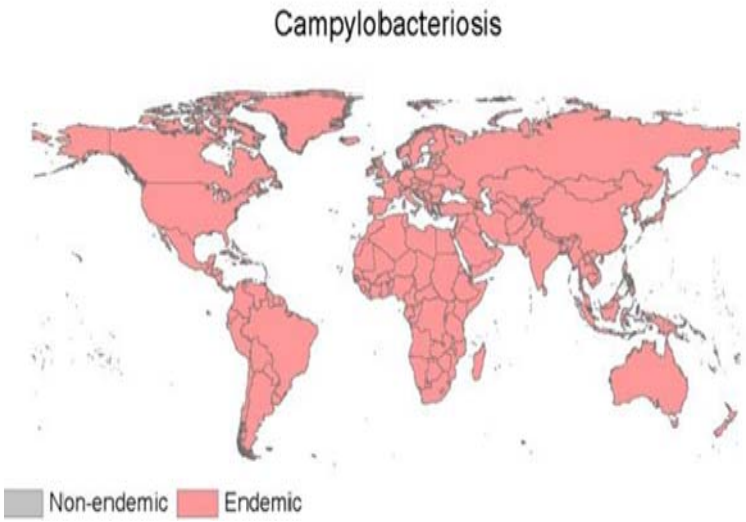
Based on the Community Zoonoses Reports of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control(ECDC) in their Community Zoonoses Reports,inthelast5years,campylobacteriosis has been the most commonly reported zoonosis in the EU followed by salmonellosis and yersiniosis **(EFSA, 2007,2010c).** In 2008, campylobacteriosis was the principal cause of zoonotic disease in humans with 190,566 reported confirmed cases **(EFSA, 2010c).** The Food Borne Diseases Active Surveillance Network (Food Net) of the Centers for Disease

Control and Prevention (CDC) in the USA estimates that in 2009, the number of reported infections and incidence per100,000 population by *Campylobacter* was 6,033 and 13.02, respectively **(Anonymous, 2010).** More than 10,000 cases of campylobacteriosis are reported each year to the CDC (approximately six cases for each 100,000 persons in the population) **Anonymous, 2010**. Reports examples of outbreaks that occurred worldwide in the recent years, mainly resulting from consumption of contaminated drinking water, raw milk, and chicken products.

Foodborne zoonoses are an important cause of morbidity and mortality worldwide; the World Health Orga- nization (WHO) estimates that over two million people die each year from diarrheal diseases mainly caused by the ingestion of contaminated foods **(WHO, 2005; EFSA, 2007).**

Novotny, *et al*.2004 stated that the human infections and intoxications with the following bacteria have been recorded: *Mycobacterium* spp., *Streptococcus iniae, Photobacterium damselae, Vibrio alginolyticus, V. vulnificus, V. parahaemolyticus, V. cholerae, Erysipelothrix rhusiopathiae, Escherichia coli, Aeromonas* spp., *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, C. perfringens, **Campylobacter jejuni**, Edwardsiella tarda, Legionella pneumophila,* and *Plesiomonas shigelloides*. **Campylobacteriosis** due to consumption of fish and related products is very rare. Incidence of Campylobacter spp. was found in fish and meat products (2.3% and 0.8%, respectively).Also in case-control studies, eating of fish has never been found a risk factor for campylobacteriosis.

Infected chicken feces may contain up to 10^9 bacteria per 25 grams, and due to the installations, the bacteria are rapidly spread to other chickens. This vastly exceeds the infectious dose of 1000-10,000 bacteria for humans. In January 2012, the UK's Food Standards Agency warned that two-thirds of all raw chicken bought from UK shops was contaminated with campylobacter, affecting an estimated half a million people annually and killing approximately 100**. (CFSPH, 2012).**



Pathogenesis of Campylobacter

Specific virulence mechanisms have not yet been clearly elucidated for *Campylobacter* spp. Probably due to the lack of pathogenesis similarity between campylobacters and other pathogens **(Dastia etal.,2010).**

Even though the disease severity may depend on the virulence of the strain as well as on the host's immune condition **(Zilbauer et al., 2008).**

Cyto lethal distending toxin(CDT) is widely distributed among Gram-negative bacteria Ge etal.,2008) and is the best characterized of the toxins produced by *Campylobacter* spp. It has been described as an important virulence factor of this pathogen **(Asakura et al., 2008).**

The individual bacteria involved were as follows: *Campylobacter jejuni* 77.3%, *Salmonella* 20.9%, *Escherichia coli O157:H7* 1.4%, and all others less than 0.1%. *Campylobacter* organism is one of the most common causes of human bacterial gastroenteritis. For instance, an estimated 2 million cases of *Campylobacter* enteritis occur annually in the U.S., accounting for 5-7% of cases of gastroenteritis. **Doyle and Erickson, (2008)**

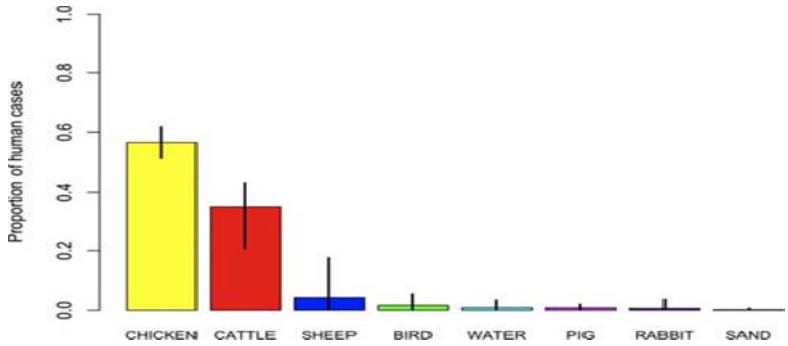


Figure 1: Estimated proportion of human cases attributable to animal and environmental sources. Error bars indicate the 95% credible interval for each source.

Warner et al., (1986) found that 23%of infected human cases with campylobacteriosis were associated with the consumption of unpasteurized milk and milk products.**in Egypt.**

Prevalence of Campylobacter spp.

Campylobacter spp. are commensal organisms routinely found in cattle, sheep, swine, and avian species. The avian species are the most common hosts for *Campylobacter* spp. Probably because of their higher body temperature **(Skirrow,1977).** Although all commercial poultry species can carry *Campylobacter* spp. The risk is greater from chicken because of the large quantities consumed **(Humphrey et al.,2007).**

Campylobacter jejuni is the leading cause of bacterial gastro-enteritis in the developed world. It is thought to infect 2–3 million people a year in the US alone, at a cost to the economy in excess of US $4 billion. *C. jejuni* is a widespread zoonotic pathogen that is carried by animals farmed for meat and poultry. *C. jejuni* is among the most common It produces an inflammatory, sometimes bloody, diarrhea or dysentery syndrome, mostly including cramps, fever and pain bacterial infections of humans, often a food borne illness. **(Ryan and Ray 2004).**. In 97% of cases, identified chicken, cattle, or sheep as the source of infection. Very few cases were attributable to campylobacter found in wild animals or the environment. The results imply that the primary transmission route is the food chain and also add new impetus to measures that reduce infection in livestock and prevent food-borne transmission **(Ryan and Ray 2004).** Some strains of ***C jejuni*** produce a cholera-like enterotoxin, which is important in the watery diarrhea observed in infections. The organism produces diffuse, bloody, edematous, and exudative enteritis(**CFSPH, 2012**)..

By modeling the DNA sequence evolution and **zoonotic** transmission of ***C. jejuni*** between host species and The environment, the assign human cases probabilistically to source populations. The population genetics approach reveals that the vast majority (97%) of sporadic disease can be attributed to animals farmed for meat and poultry. **Chicken and cattle** are the principal sources of *C. jejuni* pathogenic to humans, whereas wild animal and environmental sources are responsible for just 3% of disease.

The first full-genome sequence of ***C. jejuni*** was performed in 2000 (strain NCTC11168 with a circular chromosome of 1,641,481 base pairs **(Parkhill *et al*., 2000 and Nashwa, et al., 2013).**

2.Materials and Methods

(ii) Detection and isolation of *Campylobacter*

Identification of the isolates:(by traditional methods)

Microscopic examination

Under a phase contrast microscope for detection of motilily and S shape character of the isolated campylobacter organisms .From the suspected growth, a loopful was taken and put on clean slide and covered with cover slips. Primary examination of smears from the inoculated tubes were examined under phase contrast microscope using (400 x) magnification power for detection of characteristic actively motility and morphology of Campylobacter organisms .

Morphological identification

Suspected growing colonies onto blood agar plates were examined carefully for their morphological characters according to **Holt et al. (1994)** A single suspected colony was stained with Gram's stain to demonstrate the characteristic morphology of isolates.

Motility test

Deep stab growth

Ability of isolates to grow under microaerophilic conditions was determined by inoculating a loopful of 3 days old cultures by deep stabbing, into semisolidthiol medium, then incubated at 37°C and examined after 48 hours.

Typical growth ring test
Colonial characteristics:

Rounded, small, translucent, grey, buffy or brownish colour onto blood agar plates, typical Campylobacter species were seen after 48- 72 hours incubation at 37°C under microaerophilic conditions. Films were made from suspected colonies and stained with Gram's staining to demonstrate the characteristic features of Campylobacter organisms.
Temperature tolerance test:

The ability of isolates to grow under microaerophilic conditions at 25°C, 37°C and 42°C was demonstrated after 72 hours incubation.
Biochemical identifications: according to - **Krieg, and Holt, (1984)**

- -Catalase production test **(Laing, 1960)**
- -Nitrate reduction test **(Bryner and Frank, 1955)**
- -Hydrogen sulphide production **(Bryner et al.,(1962)**using lead acetate paper
- -Glycine tolerance test **(Chang and Oog, 1971)**
- -Sodium chloride (NaCl) tolerance test **(Taul and Klechner, 1968)**
- -Hippurate hydrolysis test **(Carter,et al.,1984)**
- -Sensitivity to nalidixic acid and cephalothin

Molecular characterization of campylobacter isolates.

The investigation of *C. jejuni* and *C. coli* infection in contaminated food and water exist and that strains are associated with different hosts (human, poultry and young animals). Therefore, we need sensitive and specific laboratory methods such as PCR and Fluorescence antibodies techniques for diagnosis of *Campylobacter* organisms cause food illness from different localities in Egypt.

1. Molecular assignment to *Campylobacter* species by PCR.[Person, and Olsen (2005)]:

DNA extracts were prepared for each isolate by boiling one bead from each Microbank tube in 0.5 ml of sterile distilled water for 20 min. Cell lysates were kept at 4°C for no longer than 14 days. Isolates were confirmed to be *C. jejuni*, *C. coli*, or *C. lari* using a previously described multiplex PCR that targeted the *hipO* and 23S. rRNA genes of *C. jejuni* and the *glyA* gene of both *C. coli* and *C. lari* **(Wang *et al.* 2002).**

Three further single-PCR assays were used to amplify the *ceuE* gene of both *C. jejuni* and *C. coli* (Gonzalez, *et al*,1997) and the 16S rRNA gene of *C. lari* (28) to confirm the results generated by the multiplex PCR assay described above **(Wang *et al.* 2002).**

MLST of *C. jejuni* isolates. Chromosomal DNA was extracted from freshly grown *C. jejuni* using a NucleoSpin tissue DNA extraction kit (Macherey-Nagel, Du¨ren, Germany) according to the manufacturer's instructions. Segments of seven housekeeping genes, *aspA* (aspartase), *glnA* (glutamine synthetase), *gltA* (citrate synthase), *glyA* (serine hydroxymethyl transferase), *pgm* (phosphoglucomutase), *tkt* (transketolase), and *uncA* (ATP synthase alpha subunit), were amplified by PCR and sequenced using protocols, primers, and reaction conditions described. Sequencing reactions were carried out using an ABI Prism 3130x genetic analyzer.

The STADEN software package **(Staden, R. 1996.)** was used to assemble sequences from the chromatograms generated by the ABI Prism 3130x genetic analyzer (Applied Biosystems), and allele numbers were assigned by comparing sequences with sequences in the public MLST profile database (http://pubmlst.org campylobacter). Sequence types (STs) and clonal complexes were also assigned by comparison with the STs and clonal complexes in the MLST database. Novel sequences and STs were submitted to the database, and new allele and ST numbers were assigned. MLST was not attempted with the *C. coli* isolates as three of the four PCRpositive *C. coli* isolates could not be revived from storage at _80°C.

2.b.Fluorescent antibody technique (as rapid method)according Nettleton and herrimg (1983):

Glass coupons coated with biofilm obtained from the water model experiment were washed gently with sterile water, fixed with acetone, and air dried. The coupons were then supported on microscope slides. A monoclonal antibody was appropriately diluted with fetal calf serum–phosphate-buffered saline (1:10, vol/vol), and 50-µl aliquots were dispensed onto each coupon. The slides were incubated in a humidified darkened box at 37°C with gentle shaking for 45 min. The slides were then gently washed in sterile water and placed in a heated cabinet (~40°C) to air dry. Fifty microliters of appropriately diluted anti-immunoglobulin G- or anti-immunoglobulin M-specific goat anti-mouse fluorescein isothiocyanate-labelled conjugate (Sigma) was then placed on each slide, and the slides were incubated as previously described. The slides were again washed gently with sterile water and air dried. Coverslips were placed over each coupon along with approximately 5 to 10 µl of mountant and fluorescence enhancer Citifluor AFI (Amann *et al* 1990 and Clive et al.1998). The slides were examined by epifluorescence microscopy (Leitz model Dialux 20EB microscope) by using a water immersion objective (magnification, ×50), a 50-W mercury lamp, a type KP 490 blue excitation filter, a type K530 barrier filter, and a type TK510/K515 dichroic beam splitting mirror. Photographs were taken by using a Leitz Vario Orthomat camera and

Ektachrome 320 tungsten color reversal film (Kodak).

(iii) Campylobacteriosis and presence of virulence factors

Based on the Community Zoonoses Reports of the European Food Safety Authority(EFSA) and the European Centre for Disease Prevention and Control (ECDC) in their Community Zoonoses Reports,inthelast5years,campylobacteriosis has been the most commonly reported zoonosis in the EU followed by salmonellosis and yersiniosis **(EFSA, 2007,2010c).** In 2008, campylobacterio- sis was the principal cause of zoonotic disease in humans with 190,566 reported confirmed cases **(EFSA, 2010c). The** Food borne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention (CDC) in the USA estimates that in 2009, the number of reported infections and incidence per100,000 population by *Campylobacter* was 6,033 and 13.02, respectively **(Anonymous, 2010).** more than 10,000 cases of campylobacteriosis are reported each year to the CDC (approximately six cases for each 100,000 persons in the population) **Anonymous, 2010**. Reports examples of outbreaks that occurred worldwide in the recent years, mainly resulting from consumption of contaminated drinking water, raw milk, and chicken products.

Foodborne zoonoses are an important cause of morbidity and mortality worldwide; the World Health Orga- nization (WHO) estimates that over two million people die each year from diarrheal diseases mainly caused by the ingestion of contaminated foods **(WHO, 2005; EFSA, 2007).**

Conclusion

Food borne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. For solving these problems must take some advices include:

- Globalization of the food supply, resulting in rapid, international distribution of raw and processed foods and exposure to duced in less well-regulated environments.
- Economic pressures to provide products as cheaply as possible, requiring large scale production and distribution processes.
- Traditional food production and handling practices that may be inappropriate in the modern production and retailing environment.
- Public and political expectations about the safety of the food supply.
- Population-health factors that may increase risk of illness, including age (the young and elderly), existing illness (e.g., cancer), inherited traits (e.g., sickle cell disease; HLA B-27 susceptibility to reactive arthritis), and depressed immunity (from AIDS, cancer treatment, transplants, pregnancy, and poor nutrition).
- New pathogens and antibiotic-resistant strains possibly related to environmental factors and changes in farming and husbandry practices.

The response to these challenges involves government, the food industry, the public health community, and the public.

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