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The Silent Threat: A journey into Campylobacter Foodborne

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Review Article

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Abstract

Campylobacter species are one of the risky human foodborne pathogens worldwide. This bacterial group poses considerable public health concern due to its emergence as a major cause of human disease and its propensity to exhibit antibiotic resistance. *Campylobacter jejuni* and *Campylobacter coli* are usually regarded as two most significant enteropathogens. They are characterized by its high capacity to colonize and persist in a broad scope of animal species and environments, making it difficult to be managed or controlled. This article analyzed the historical and growing significance of Campylobacter species as well as their features in human illness, their pathogenesis and reservoirs.

Keywords: *Campylobacter spp, foodborne pathogens and Campylobacter jejuni.*

Introduction

Campylobacteriosis is one of most serious zoonotic foodborne pathogenic human infections worldwide (**Mortada, 2021)**. The consuming of contaminated undercooked poultry flesh is implemented in most human issues of campylobacteriosis, particularly *Campylobacter jejuni* subsp. *jejuni* (*C. jejuni*); 90% (**Matthew, 2020).** An insufficient or poorly cooked meat of animal origin and unpasteurized milk or its products and food that had been cross contaminated prior to human consumption were recorded also as other routes of infection in human campylobacteriosis **(Skarp, 2016)**. Human illness is usually self-limiting even though it is severe illness. Diseased persons with Campylobacter bacterial infections could exhibit diarrhoea that sometimes tinged with blood (bloody stools), abdominal pain and malaise (might persist for 1-11 days). *Campylobacter jejuni* is the most species often recovered from humans (84%) and poultry (81%), while *C. coli* is always found in swine (100%) **(Saenz *et al.* 2000).**

Moreover, Campylobacter spp. can speedy colonize 95% of the birds in a commercial broiler flock. Just, after exposure to a sole infected bird, the flock remains carrier of the infection until it is ready for market (**Stern *et al*., 2001)**. However, early protection against campylobac-

teriosis with maternally derived antibodies in the commercial broiler flocks could occur and Campylobacter negative till 2 weeks of age. Campylobacter pathogens colonize primarily in the bird ceca where its bacterial load could reach up to 10^7-10^9 CFU/g **(Cawthraw *et al.*, 2010).** In 2010, a report mentioned that campylobacteriosis was the leading zoonotic illness recorded in the European Union (EU) with 212 064 asserted cases, **Anonymous (2010)**. Furthermore, a marked rise in infection cases has been noticed since 2006. Campylobacter is extensively distributed in poultry; although, cattle, sheep, pigs, and pets may also be a source of these pathogens. Fresh chicken meat is a prevalent source of Campylobacter, and within the European Union, the percentage of contaminated chicken flesh sold in stores ranged widely, from 3.1% to 58.8%, relying on the country.

History:

It is considered that Theodore Escherich had documented the first report providing Campylobacter in 1886 and described the organism as non-culturable spiral-shaped bacteria **(Vandamme *et al.,* 2010),** subsequently also detected in kittens. After that, Campylobacter was recognized in 1906 when two British veterinarians recorded the existence of "large numbers of a peculiar organism" in the uterine mucosa of a pregnant ewe. Persuading this, **McFaydean and Stockman (1913)** recognized Campylobacter in a fetal tissue of aborted ewe inspected specimen. **Sebald and Véron (1963)** firstly determined the genus Campylobacter through the isolation of Campylobacter from blood specimens of diarrheic children, differentiating it from Vibrio spp. In 1972, the Belgian microbiologists mentioned that selective growth media permitted many laboratories to examine easily the human stool specimens for campylobacteriosis diagnosis. Since 1980, Campylobacter species have been recognized as common human pathogens.

Taxonomy and classification:

The Campylobacteraceae is the biggest and most assorted family in the phylogenetically discrete Epsilon proteobacteria (Epsilon proteobacteria is the phylogenetically-distinct lineage within the Proteobacteria) **(Garrity, 2005).** Genus Campylobacter involved more than fourty species and subspecies, of which the most important ones are:

1. *Campylobacter fetus:* which includes three subspecies; subsp. *fetus*, subsp. *venerealis* (**Sebald and Véron, 1963)** and the more recently identified subsp. *testudinum* **(Fitzgerald *et al.*, 2014).**

2. Campylobacter jejuni (that diversed into two subspecies: subsp. *jejuni* described by **Jones *et al.*, (1931) then Howey *et al.*, (1990)** and subsp. *doylei* **(Steele and Owen, 1988).** Also, less common other *Campylobacter* species were also documented; *C. sputorum* (subsp. *Sputorum,* and subsp. *bubulus*) **(Véron and Chatelain, 1973),** and *C. ureolyticus* **(Jackson and Goodman, 1978).**

Currently, there are numerous variant *Campylobacter* species belong to this family; *C. coli*, *C. jejuni*, *C. fetus*, *C. lari*, , *C. hyointestinalis*, *C. jejuni* subsp. *doylei, C. sputorum* biovar paraureolyticus, *C. concisus C. curvus*, , , *C. helveticus*, , *C. gracilis C. hominis*, *C. insulaenigrae C. lanienae*, *C. mucosalis C. rectus*, *C. showae*, and *C. upsaliensis*. Additionally, there are new species determined; *C. avium* sp. nov., *C. cuniculorum* sp. nov., *C. canadensis* sp. nov., *C. subantarcticus* sp. nov., , *C. peloridis* sp. nov., *C.* ureolyticuscomb. nov., *C. troglodytis* spp. nov. and *C. volucris* sp. nov. **(Inglis *et al.*, 2011).**

Growth and survival:

It was noted that Campylobacter spp. was ideally Gram-negative, non-sporulated, spiral shaped or S-shaped bacterium (0.5–5 µm long and 0.2–0.8 µm wide), with sole polar flagellum at one or both ends, awarding a peculiar corkscrew-like motility. Almost, these bacteria need micro-aerobic circumstances, but some species also grow anaerobically or aerobically. Interestingly, some species, especially *C. coli, C. jejuni* and *C. lari* are thermophilic; growing at 42°C as optimum temperature. They neither oxidize nor ferment carbohydrates. Campylobacter can colonize mucosal surfaces, frequently the intestinal tract, and occasionally genital tract of most mammalian and avian species. Notably, *C. jejuni* comprises two subspecies (subsp. *jejuni* and subsp. *doylei*) that can be distinguished on the basis of numerous phenotypic tests (safranine, sodium

fluoride, selenite reduction, and nitrate reduction) as well as *C.* subsp. *doylei* does not grow at 42°C and is less frequently isolated than subsp. *jejuni* **(Garrity *et al.*, 2005)**.
The constancy level of Campylobacter is due to their propensity to adhere or captured in the skin surface hindering the chances of the removal of Campylobacter during carcass washing. This tendency of Campylobacter spp. also offered enough protection against environmental stress that was occurred during chilling, heating rather than exposure to chlorinated water. Campylobacter haven't the ability to multiply in the processed plant, as the least growth temperature is 32-35°C while the convenient is 37-42°C. Their growth outside the intestine needs a minimized conc. of atmospheric O_2 and preferably 10% CO_2. It's paradoxical that Campylobacter; a difficult-to-grow pathogen, flourishes in circumstances similar to those within a host but is easily harmed by environmental stressors such as atmospheric oxygen heat, high salinity oxygen, heat , dehydration, and UV radiation. Despite being found in diverse environments, such as acidic grapefruit juice at refrigerated temperatures and water sources **(Ferro *et al.*, 2018)**. For example, Rollins and co-authors demonstrated the survival of Campylobacter in water for over 120 days **(Rollins and Colwell, 1986).**

Reservoirs and sources
Campylobacter bacteria are ubiquitous in the environment and are observed in most regions of the world. They can be isolated from wild and domesticated warm-blooded animals (e.g., mammals and avian species), food commodities (e.g., red and white meat, dairy products and vegetables) and environmental samples (e.g., soil, fresh water and sea water) **(Facciolà *et al.*, 2017)**. Generally, Campylobacter reservoirs are mainly found in animals (e.g., cattle and poultry) and spread via contaminated sources and transmission routes. Major contaminated sources by Campylobacter include environment and water. Routes of transmission occur when food, tools and humans come in direct contact with an infected animal or a contaminated source. Thus, risk factors of campylobacteriosis include the consumption and handling of foods commonly associated with Campylobacter **(Newell *et al.*, 2017**). **El- Sisi and Ibrahim (2002)** isolated Campylobacter spp. from 141 (63.8%) out of 221 birds and rabbit examined samples. The highest prevalence was recorded in chickens (44.1%) followed by ducks (20.5%), rabbits (16.1%), pigeons (12.9%), and quails (6.3%). The highest rate of *Campylobacter* spp. isolation was from cecal contents (40.6%), liver (30.8%), spleen (19.9%) and heart blood (8.6%). For humans or consumers, **Kelli *et al.*, (2002)** reported that the main route of Campylobacter infection in humans is thought to be through contact with and consumption of poultry or its products. Campylobacter had been recovered from as much as 75% of the live poultry population and from as many as 80% of processed broiler flesh specimens commercially sold. Numerous potential sources and vectors of contamination that have been studied involve from parent to offspring via egg transmission, pre-existing contamination in rearing facilities, contaminated water, feed, litter, hatchery pads, human workers, small animals on the farm, rodents, and flies.

Also, Campylobacter bacteria have been transmitted to humans by a large variety of food-related vehicles including drinking water, red meat (e.g., pork and beef), shellfish, ready-to-eat sandwiches , as well as fruits and vegetables (e.g., lettuce, spinach, radishes, and peas). Most cases associated with shellfish, fruits and vegetables are likely due to cross-contamination of water with the faeces of wild avians (e.g., seagulls) or fertilizers and soils that were in contact with infected warm-blooded animals (**Newell *et al.*, 2017).**
Wieezorek *et al.*, (2013) reported that following to the near report of European Food Safety Authority (**EFSA, 2007**), the most prevalent zoonotic illness reported among peoples in the EU in 2008 was Campylobacter infection, with incidences of 40.7 infections per 100,000 people**.**
A study in Alexandria, Egypt, analyzing fecal samples from 1,079 healthy children and 880 diarrheic children (average age 9 months), revealed that Campylobacter spp. was significantly more common in children with diarrhea (17.2%) than in healthy children (6.4%). Cam-

pylobacter was also more frequently found in diarrheal cases than Salmonella (3%), Shigella (2%), or other bacterial pathogens (1%) **(Pazzaglia *et al.* 1995)**.

Infectivity and symptoms

Campylobacteriosis is a zoonotic disease that is caused by *Campylobacter* bacteria affecting regular absorptive and secretory functions of the human gastrointestinal tract after colonizing the ileum and colon **(Backert *et al.*, 2017**).

The characteristic symptom of campylobacteriosis is acute watery and/or bloody diarrhea. Other symptoms include fever, abdominal pain, vomiting and dehydration. Symptoms commonly occur within 1–5 days after a Campylobacter infection. Most 3 Campylobacter infections are self-limiting, particularly in healthy individuals, and symptoms typically disappear within 3 weeks **(Schielke *et al.*, 2014)**.

Common risk groups for campylobacteriosis are predominantly immunocompromised individuals **(Kennedy, 2004)**, children beneath the age of five years and older people over the age of 74 years **(Lévesque *et al.*, 2013**). Only a few hundred bacterial cells of *Campylobacter* are enough to be infective and bypass the colonization resistance barrier in humans and lead to campylobacteriosis **(Backert *et al.*, 2017)**. Otherwise, the dose related to causing an infection has been shown to strongly depend upon the specific Campylobacter strain **(Teunis *et al.*, 2018)**.

Specific therapy is not often necessary, except to supersede electrolytes and water that were lost during grave diarrhea, while for treating invasive cases and the carrier state, antimicrobials might be required. Severe cases of Campylobacter infections can be burdened by chronic complications known as sequelae. Rare post-infectious and extra-intestinal complications include bacteremia, meningitis, 4 endocarditis, cellulitis, peritonitis, and brain abscesses. Campylobacteriosis can also affect the joints inducing post infection reactive arthritis, also impact the nervous system causing Guillain–Barré syndrome (GBS) **(Endtz, 2020)**. GBS is an immune-mediated flaccid paralysis and causes symptoms, such as hypo- or areflexia and paresthesia, as well as acute or subacute correspondance ascending weakness of the limbs **(Backert *et al.*, 2017** and **Endtz, 2020)**. The fatalities in developing countries are hypothesized to be significantly higher due to limited access to the optimal treatment **(Facciolà *et al.*, 2017**). Many cases are self-limiting and disappear within 5 6 months, but it has been estimated that up to 63% of patients develop a specific chronic form of reactive arthritis **(Carter, 2006)**

Characteristic features of *Campylobacter* species:

1. Morphology:

Old cultures of the bacterium might appear coccoid or spherical bodies. Both ends of the cell are enclosed by a multilayered polar membrane situated beneath the cytoplasmic membrane. Their flagella might be two to three times the length of the cells. There are exceptions; species have multiple flagella (*Campylobacter showae*) while *Campylobacter gracilis* is non-motile species **(OIE, 2008 and Vandamme *et al.*, 2015)**.

2. Cultural character of *Campylobacter* spp.:

The isolation and detection of different Campylobacter species in foods and food-animal matrices needed different growth requirements. It relies on the kinds of media and laboratory isolation method employed. Primary isolation of this pathogen usually needs the use of non-selective media, selective filtration and incubation at 37°C.

Multiple prior studies discussed many trials for isolation of Campylobacter spp. In 1992, **Albert *et al.* (1992)** used blood-free medium (BFM) and compared it with filtration technique on 5% sheep blood agar that was overlaid with a 0-65 μm pore size of Sartorius membrane filter to isolate Campylobacter spp. from 676 diarrheic stool samples in Australia. The inoculated BFM was then incubated at 42 °C in anaerobically in a specific jar with the aid of catalyst under a microaerophilic atmosphere which was generated with a Campylobacter gas-generating sachet. Then, the plate was inspected after 48 hours and, if no growth of Campylobacter was observed, the plate should be discarded. Initially, the scientists suggested that prolonged incubation of Campylobacter cultured BFM plates over 48 hours; did not affect their isolation rates. However,

the cultured Campylobacter plates were better to be re- examined again after 48 hours and daily till 5 days.

Moreover, **ISO 10272 (1995)** declared the isolation of Campylobacter could be by three methods; two of them were based mainly on pre-enrichment while the third focused on the direct plating. Pre-enrichment is relied on either Park and Sanders or Preston broth. The specimens are cultured to nine folds (volume or weight /volume) and then incubated at 42°C beneath microaerophilic atmosphere for forty eight hours. The direct plating protocol comprises spread-plating of an aliquot of specimen to 2 agars in parallel. Incubation of agar plates in a microaerophilic atmosphere was achieved at 42°C for 24 - 72 hrs. For all 3 protocols, there is a needing for assertion of thermotolerant Campylobacter. Selection of 5 characteristic colonies from each selective agar plate was achieved for Gram stain examination. Microscopic inspection of wet mount specimens was performed to examine motility. Also, the isolates are examined for oxidase production and their capacity to grow at 25°C. Lastly, isolates were tested by culturing in Brucella broth as non-selective medium and incubated in microaerophilic conditions for 2 – 5 days. In addition, Campylobacter could be cultured on Skirrow medium, Columbia blood agar and charcoal cefoperazone deoxycholate agar (CCDA) that covered with a 0.65 µm pore size membrane filter (**Piersimoni *et al.*, 1995).**

3. Biochemical characteristics of Campylobacter species:

The identification scheme of Campylobacter spp. is based mainly on their characteristics on catalase, oxidase, hippurate hydrolysis and indoxyl acetate hydrolysis, susceptibility to cephalothin and nalidixic acid **(ISO, 2006)**. *C. jejuni* is the only species that could be differentiated from other Campylobacter species. It is the only hippurate-positive Campylobacter species however, some strains of *C. jejuni* were found hippurate-negative. Sensitivity of Campylobacter species to nalidixic acid became misdiagnosed nowadays in the identification scheme as many strains of *C. jejuni* and *C. coli* became nalidixic acid-resistant. Also, many nalidixic acid-sensitive genogroups of *C. lari* were recorded. So, the data should be asserted using specifically determined positive and negative controls. Moreover, *C. jejuni* could hydrolyse hippurate, and indoxyl acetate and reduce **(OIE, 2008)**.

Molecular advanced techniques for rapid detection of Campylobacter:

Nucleic acid-based technologies have been commonly used in the last years to detect specific DNA or RNA sequences. Campylobacter DNA could be sequenced, magnified or amplified then gel displayed, or might be quantitatively determined or subjected to the molecular identification **(Ghatak *et al.*, 2020)**. The Polymerase chain reaction (PCR) approach is fast, accurate, highly discriminative and relatively simple to distinguish between Campylobacter species (**Denis *et al.*,2001)**. Virulence determinants in *C. jejuni* and *C. coli* are accurate aids to evaluate the possible risk of poultry as a concern of Campylobacter infection **(Melo *et al.*, 2013)**. DNA sequencing also makes rapid and precise identification of Campylobacter species but also with ability to expose the epidemiological traits of this species. These aids also enable researchers to create data generation that can be published through web-based databases and utilized in phylogenetic maps of this bacterial species **(Negahdari *et al.*, 2016)**. Real time or quantitative PCR (qPCR) are two synonyms terms the qPCR technology **(Ghoneim *et al.*, 2020)**. Certainly, the adopting for the particular gene target in the routine diagnosis improved greatly the perception of the epidemiology of Campylobacter infection in poultry and human and its public health concern.

Control measures for campylobacteriosis:

Increasing the biosecurity and general hygiene standards is the most effective intervention on-farm to prevent Campylobacter infection from being introduced into avian farm **(D'angelantonio *et al.*, 2021 and Dogan *et al.*, 2022).** It is substantial to reduce contamination of poultry rearing houses via installing hygienic barriers within the internal and external environments, such as monitoring the entry of farm personnel. Also implementation of sharp hygienic routines such as hands' washing and sanitization, changing boots and coveralls prior

entering, have been displayed to be efficient, but these barriers have frequently been found to be broken. Breeding poultry in a free-range system has a much greater risk of infection parallel to conventional output and therefore rising difficulties in control (**Humphrey *et al.*, 2007**).

The incidence of Campylobacter in broilers had been impacted by acidifying litter treatments, since it reduced the litter circumstance's hospitability to Campylobacter as well as other foodborne pathogens like Salmonella **(Chinivasagam *et al.*, 2020 and Hwang and Singer, 2020)**. In addition, broiler chicks had been immunized against Campylobacter demonstrated the most promising results **(Helmy *et al.*, 2022)** especially following intramuscular injection. It was proved that immunization with Campylobacter vaccine (which is made from multiplication proteins that were exhibited on the surface of *C. jejuni*) could reduce in the degree of infection with a 2 log in 20-day-old hens **(Neal-McKinney *et al.*, 2014).** Probiotics could significantly reduce the prevalence of Campylobacteriosis **(Taha-Abdelaziz *et al.*, 2019** and **Khan *et al.*, 2020)**. Similarly, *Bacillus subtilis* PS-216 exhibited significant anti-bacterial activity against Campylobacter spp. **(Šimunovi´c *et al.*, 2022)**.

At the current period, no established on-farm therapies shown to be effective in decreasing the incidence of campylobacter infections in broilers as mentioned by **(Beterams *et al.*, 2023).** The novel methods as bacteriocins, vaccinations, and probiotics were employed also to lessen the colonization of campylobacter on farms and in slaughterhouses.

Conclusion

Globally, raw or under-cooked chicken and their outputs are the main concern of human campylobacteriosis. The frequency of antibiotic-resistant Campylobacter spp. has increased in tandem with the growth in Campylobacter-related diseases. Because Campylobacter is the primary source of human illness, it is imperative to initiate novel natural antimicrobial therapies in conjunction with suitable hygiene and biosecurity policies at the farm level to minimize the colonization of Campylobacter spp. in commercial poultry flocks. Certain immunizations and some feed additives are required to combat the most virulent factors of Campylobacter which could strengthen its pathogenesis and survival in the host. Additionally, implementing of the HACCP regulations, inspecting and enforcing chicken meat, forming stakeholder groups, and providing a good training for food handler or workers will minimize the risk of campylobacteriosis human infections.

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