

Review Article

Campylobacter species: New insight, clinical diagnosis and laboratory approach in Nigeria

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ABSTRACT

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Campylobacter, a leading cause of gastroenteritis globally, has seen increased prevalence in recent years, affecting both industrialized and developing nations. While *Campylobacter jejuni* and *C. coli* are responsible for most cases, understanding the disease mechanisms, transmission, and evolution of these lesser-known species is crucial due to emerging pathogens. High-throughput sequencing has made numerous whole-genome sequences accessible, allowing for in-depth pathogenomic studies on various species like *C. fetus* and *C. concisus*. These investigations have unveiled genomic traits associated with pathogenicity and shed light on the evolutionary mechanisms shaping their genomes, offering new clinical microbiology applications. To tackle these pathogens effectively, deeper insights into genome dynamics and pathogenicity evolution in emerging *Campylobacter* species are urgently needed. This review compiles existing knowledge and outlines future research directions in this field. *Campylobacters* are Gram-negative, non-spore forming, curved or spiral rods. The "thermophilic group" relevant to the water sector comprises *C. jejuni*, *C. coli*, and *C. upsaliensis*. Human *Campylobacter* infection primarily presents as acute diarrhea, with flagellar and antibiotic resistance genes identified as key virulence factors. Many *Campylobacters* are found in sewage and treated sewage effluents, with lower levels in surface waters. Effective epidemiological investigations into *Campylobacter* prevalence combine phenotypic and genotypic tests. Despite sensitivity to oxygen, *Campylobacters* exhibit robust survival mechanisms, potentially involving biofilm formation. Approaches used to inactivate coliforms are considered effective against *Campylobacter*. This review provides a comprehensive overview of the field, stressing the importance of further research into these pathogens.

INTRODUCTION

The most common bacterial group, *Campylobacter*, causes food-borne gastroenteritis in people all over the world. There were around 845,000 instances per year in the US, according to a 2011 data from the Centers for Disease Control and Prevention (CDC). Even while these conditions rarely result in death, they can do so if an immunocompromised person contracts them. The two types of bacteria most frequently linked to human *Campylobacteriosis* are *Campylobacter jejuni* and *C. coli*. In addition, a number of additional *Campylobacter* species have been linked to illness. In Ireland and throughout Europe, *Campylobacter* species is the most frequent bacterial cause of gastroenteritis. Microaerophilic, Gram-negative, spiral-shaped cells with corkscrew-like movement are seen in the *Campylobacter* species. In many nations, including Ireland, they are the most frequent cause of bacterial gastroenteritis, and they usually spread to people through

food. The most common species linked to human sickness is *Campylobacter jejuni*, along with *Campylobacter coli* and *Campylobacter lari*⁴.

Foods with an animal origin usually include isolated *Campylobacter* species. One of the most prominent sources of *Campylobacter* spp. is considered to be poultry, which also plays a key role in the pathogen's transmission to people. One of the most prevalent bacterial illnesses in humans is campylobacter. They cause both systemic and diarrhoeal diseases. Enteric *Campylobacter* infections cause an inflammatory, occasionally bloody diarrhea or dysentery condition in industrialized areas⁵. The most frequent cause of community-acquired inflammatory enteritis is typically *Campylobacter jejuni*. In underdeveloped areas, diarrhea could be watery.

Since the early 20th century, campylobacter species have been thoroughly characterized in veterinary medicine. The significance of *Campylobacter* species as gastrointestinal infections in humans, however, wasn't widely acknowledged until the 1970s. In the 1970s and 1980s, as laboratory testing and capacity increased, *Campylobacter* species was swiftly identified as one of the major causes of bacterial enteritis in humans globally⁵. There was indications that the data were reflecting an actual upward trend above and above the apparent increase that would be anticipated to occur with enhanced detection and surveillance methods as reported incidence rates grew throughout the later decades of the twentieth century.

In many industrialized countries today, *Campylobacter* infections are the most often reported cause of bacterial gastroenteritis. In poor countries, *Campylobacter* infections are a significant cause of bacterial gastroenteritis in young children. The true incidence almost certainly exceeds the number of reported cases by a significant margin⁴. Although some nations have recently seen a decrease in the incidence of *Campylobacter* disease, there is concern that the human immunodeficiency virus (HIV) epidemic may significantly increase the disease burden of *Campylobacter* in developing nations. Concurrent HIV infection is known to increase the incidence risk and severity of *Campylobacter* gastroenteritis³.

There are other public health issues associated with these organisms in addition to the significant disease burden of *Campylobacter* gastroenteritis. Recent studies have emphasized the crucial part that *Campylobacter* infection plays in the pathogenesis of reactive arthritis and other post-infectious disorders including Guillain-Barré syndrome (GBS), a devastating neurological condition.

Given the pervasive contamination of the food supply chain by *Campylobacter* and the numerous ways that humans can become infected through food and water, campylobacter infection is particularly relevant to the food production industry⁶.

In a subsequent section of this text, a number of methods for disease prevention and management are discussed, however it is obvious that managing *Campylobacter* infection is a difficult undertaking. The condition known as campylobacteriosis is brought on by a campylobacter infection. After bacterial infection, illness symptoms often appear 2 to 5 days later, but they can also appear up to 10 days later. Diarrhoea (often bloody), abdominal pain, fever, headache, nausea, and/or vomiting are among the most typical clinical signs of *Campylobacter* infections. Typically, the symptoms last 3 to 6 days. Campylobacteriosis is an uncommon cause of death that typically affects extremely young children, elderly patients, or people who are already ill with another deadly illness like AIDS. Numerous complications have been documented,

including bacteraemia (the presence of germs in the blood), hepatitis, pancreatitis (liver and pancreas infections, respectively), and miscarriage. Reactive arthritis, a painful inflammation of the joints that can last for months, and neurological conditions like Guillain-Barré syndrome, a paralysis that resembles polio and in a small percentage of cases can cause severe neurological and respiratory dysfunction, are examples of post-infection complications¹.

The discovery of a curved-shaped bacterium that caused abortion in sheep and cattle by McFaydean and Stockman in 1913 led to the discovery of the first known *Campylobacter* infection. When Smith and Taylor recovered the same bacteria from the fetal secretions of a cow in 1919, they gave it the name *Vibrio fetus*. Then, in 1973, Véron and Chatelain reclassified *V. fetus* as *Campylobacter fetus* and created the genus *Campylobacter*. In addition to its long history and established significance as a veterinary pathogen since the turn of the 20th century, *C. fetus* was later discovered to be the primary culprit in human bloodstream infections. But it wasn't until the invention and widespread use of selective media for the isolation of *Campylobacter* from stool samples in the early 1980s that the major importance of campylobacters as a primary cause of human disease was discovered. The species *C. jejuni*, which is a leading cause of bacterial gastroenteritis in humans and has an even higher prevalence worldwide than extremely well-known pathogens that cause acute gastrointestinal infections like *Escherichia coli*, *Shigella*, or *Salmonella*, is currently the most important species within the genus. *C. coli*, a close relative of *C. jejuni*, is responsible for 1 to 25% of all diarrheal illnesses linked to *Campylobacter*. The remaining species of the genus have received much less attention, but thanks to routinely applied molecular techniques, improved culture media, and improved growth conditions, a growing variety of *Campylobacter* species distinct from *C. jejuni* and *C. coli* were described and identified as relevant pathogens for people and other animals⁸.

The rise of high-throughput sequencing as a popular tool for studying the microbial world coincided with the clinical recognition of many of these emerging *Campylobacter* species, which stoked interest in using whole-genome sequencing and comparative genomics to understand how emerging campylobacters cause illness, spread, and evolve. Nearly 20 years after the publication of the first *C. jejuni* whole genome sequence, public databases now provide access to thousands of *C. jejuni* and *C. coli* genomes. As a result, comparative genomics studies that once only contained a few genomes can now include hundreds to thousands of them because of the growing amount of whole-genome sequences. Our knowledge of the biology of nonclassical *Campylobacter* infections is being hampered by the slow and fragmented pace at which genomic data for new *Campylobacter* species is becoming available. Using comparative genomics as a lens, we summarize the most recent research on emerging campylobacters in this review. We also discuss how these data are shedding light on fundamental *Campylobacter* pathobiology and its applications in clinical microbiology. Finally, we highlight upcoming challenges in the field, such as the need for additional work to reduce sequencing bias in favor of well-known species. This review serves as a thorough resource for scientists studying *Campylobacter* genomes and emerging pathogens with the goal of integrating current information and addressing unresolved issues in the study of developing *Campylobacter* pathogens⁶.

Gram-negative bacteria belonging to the *Campylobacter* genus can have morphologies that range from spiral to rod to curved, depending on the species. Others feature a single polar flagellum or bipolar flagella, while other species are aflagellate. There are 25 species in the *Campylobacter* genus, along with two provisional species and eight subspecies. In addition to being present in

humans and domesticated or wild animals like cattle, birds, reptiles, and shellfish, *Campylobacter* species have a wide ecological distribution. The most common cause of gastroenteritis worldwide is *C. jejuni*. Other *Campylobacter* species, such as *C. coli* and *C. fetal*, are recognized as being harmful to both people and animals. The term "emerging *Campylobacter* species" refers to a number of other species, including *C. concisus*, *C. ureolyticus*, and *C. lari*, which are recognized for their growing significance in human and animal infections. In this chapter, we discuss the epidemiology, genetic traits, attachment and invasion mechanisms, toxin generation, glycosylation, capsular polysaccharide production, biofilm development, and profile of antibiotic resistance of the *Campylobacter* genus. We also provide an overview of the use of animal models and host immune responses in understanding these species' pathogenesis⁹.

Members of the *Campylobacteriaceae* family include campylobacters. These bacteria can have a spiral, S, or curved shape and are Gram-negative bacilli. The majority move in a distinctive corkscrew pattern, which is controlled by polar flagella. At either end of the cell, a cell may have a single polar flagellum or two¹⁰.

Campylobacter species need a microaerobic environment with 3 to 5% oxygen and 5 to 10% carbon dioxide for growth. The ideal temperature for most species, which are thermophilic, is 42°C¹².

CURRENT CAMPYLOBACTER TAXONOMY

To date, the genus *Campylobacter* consists of 32 officially described species and 9 subspecies, namely, *C. avium*, *C. blaseri*, *C. canadensis*, *C. coli*, *C. concisus*, *C. corcagiensis*, *C. cuniculorum*, *C. curvus*, *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. fetus* subsp. *testudinum*, *C. geochelonis*, *C. gracilis*, *C. helveticus*, *C. hepaticus*, *C. hominis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. hyointestinalis* subsp. *lawsonii*, *C. iguaniorum*, *C. insulaenigrae*, *C. jejuni* subsp. *jejuni*, *C. jejuni* subsp. *doylei*, *C. lanienae*, *C. lari* subsp. *lari*, *C. lari* subsp. *concheus*, *C. mucosalis*, *C. ornithocola*, *C. peloridis*, *C. pinnipediorum* subsp. *pinnipediorum*, *C. pinnipediorum* subsp. *caledonicus*, *C. rectus*, *C. showae*, *C. sputorum*, *C. subantarcticus*, *C. troglodytis*, *C. upsaliensis*, *C. ureolyticus*, and *C. volucris*. These species cluster in five discrete phylogenetic groups, which all contain pathogenic microorganisms, highlighting the clinical relevance of the whole genus. Despite this scenario clearly reflecting the taxonomic diversity and the widespread presence of pathogenic lineages in the genus *Campylobacter*, not a single genome is available for some species, like *C. canadensis*, *C. troglodytis*, and *C. mucosalis*. Also, for many others, including *C. volucris*, *C. peloridis*, *C. rectus*, *C. insulaenigrae*, *C. hominis*, *C. helveticus*, *C. cuniculorum*, *C. corcagiensis*, *C. ornithocola*, and *C. avium* (31% of the genus), only a single representative genome per species is available. Importantly, when we exclude *C. jejuni* and *C. coli*, 13 out of the remaining 30 species (43%) have been at least sporadically reported to be the causative agent of infections in humans and/or other animals, and many of them are frequently associated with diverse clinical presentations, such as invasive blood infections, periodontal infections, abscesses, meningitis, diarrhea, or gastroenteritis. The lack of sufficient genomic information on the causative agents of these infections prevents the exploration of intraspecific genetic variability and patterns of genomic evolution. Consequently, relevant information about how a vast number of emerging *Campylobacter* species cause disease and transmit between hosts is

currently unavailable. However, several groups have made a considerable effort to generate whole-genome sequences for some emerging campylobacters whose relevance for public health is frequently underestimated, uncovering genomic features that represent valuable contributions to understanding the disease biology and epidemiology of these microorganisms. Thus, these cases are discussed for each individual species that have deserved attention from the field of comparative genomics¹⁵.

Phylogenetic relationships between described *Campylobacter* species. A phylogenetic tree of *Campylobacter* species dividing the genus into five distinct groups, namely, the *C. fetus* group, *C. jejuni* group, *C. lari* group, *C. concisus* group, and *C. ureolyticus* group, is shown. Names were assigned by considering the most clinically relevant species within each group¹¹.

The genus *Campylobacter* was created in 1963, but it required the development and refinement of isolation techniques in the decades that followed for members of the genus to be readily obtained in pure culture. In addition, the lack of biochemical activity shown by species of *Campylobacter*, which had hindered their classification, was overcome by the rapid development of analytical techniques, many DNA based, late in the twentieth century. In 1984, *Bergey's Manual of Determinative Bacteriology* listed only five species of *Campylobacter*. In 1991, a major taxonomic reorganization of campylobacters and related organisms was undertaken, based in part on DNA hybridization studies. This led to the genus *Campylobacter* being assigned to the newly created order *Campylobacterales*, in the class *Epsilonproteobacteria*. *Campylobacterales* also included the genera *Arcobacter* and *Sulfurospirillum*. In 1996, a probability matrix for the identification of campylobacters (and related organisms) was published that listed 13 species of *Campylobacter* with several subspecies also being described. Over the following years, the number of recognized species has more than doubled. However, none of the new species appeared to affect humans as significantly as those described in 1984.

Because of the perceived difficulty in culturing campylobacters, noncultural detection methods, based on DNA extraction and analysis, were applied to sample matrices suspected of harboring undetected species. Human feces from asymptomatic adults yielded positive results for *Campylobacter* DNA and led to proposals that as-yet-undiscovered campylobacters could be present in humans as commensal organisms, as was known to happen in birds. However, it was later established that some *Campylobacter* spp., such as *Campylobacter rectus*, could colonize the human buccal cavity, and that these organisms could therefore be present in the lower gastrointestinal (GI) tract as itinerants, and give rise to the positive results for these members of the genus⁴.

As genetic analyses became mainstream tools to identify organisms, and the physiological requirements of campylobacters were better understood, new species were identified and the relationships between species were better defined. The main causes of human illness associated with foods are *C. jejuni* and *C. coli*, but when stool samples are analyzed, medical laboratories normally identify isolates only to the level of genus. Differentiating these two species was based on the hippurate hydrolysis test because *C. jejuni* can perform this reaction whereas *C. coli* cannot. To avoid false-negative reactions, this test must be conducted with care, but it can be replaced by polymerase chain reaction (PCR)-based testing targeting the hippuricase gene⁹.

Fig. 1-7 Different Campylobacter species



Fig 1 :

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Fig 2 :

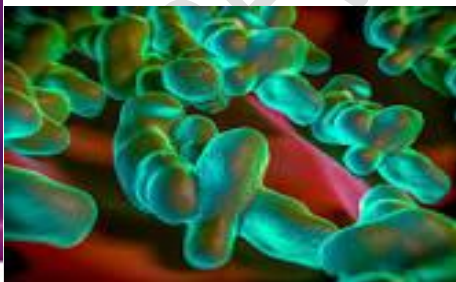


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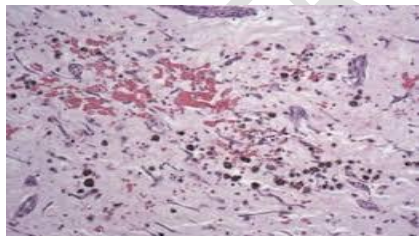


Fig 4 :



fig 5 :



Fig 6:



fig 7 :

(Olsen, et al., 2001)

EPIDEMIOLOGY

Campylobacter is recognized to be worldwide in distribution. In developing countries, *Campylobacter* is a significant bacterial cause of diarrhea in children younger than 2 years of age, yet it rarely occurs in developing nations in older children and adults. When infection does occur in the population older than 2 years of age, it tends to be asymptomatic. It is likely that patients in these countries are infected with *Campylobacter* early in life and then develop immunity, thus making **asymptomatic infection** more typical in older children and adults⁵.

In the industrialized world, most patients infected with *Campylobacter* develop symptoms. The number of *Campylobacter* infections in these countries is now recognized to be quite high, with some studies finding this organism to be the most common cause of bacterial diarrhea. *Campylobacter* tends to infect people in two distinct age groups: children in the first year of life and young adults. *Campylobacter* spp. is the most common cause of bacterial enteric infections in the United States, causing an estimated 2 million infections annually¹¹.

Campylobacter may be spread by direct contact or through contaminated sources of food and water. Milk, meat, and eggs, especially if undercooked, have been implicated in outbreaks. These sources may be contaminated from human fecal shedding, or the organisms may be harbored in the asymptomatic farm animals. *Campylobacter* is commonly spread among populations of children in day-care centers. A population-based case-control study showed that risk factors for campylobacteriosis were drinking well water, eating fruits and vegetables prepared in the home, having a pet in the home with diarrhea, visiting or living on a farm, riding in a shopping cart next to meat or poultry, and traveling outside of the United States. Infants with campylobacteriosis were less likely to be breast-fed or to be in a household where hamburger was prepared¹³.

Again, *Campylobacter* infection is a worldwide anthroponosis, with *C. jejuni* probably the most important bacterial causative agent of infectious diarrhea in humans. *C. jejuni* and *C. coli* live predominantly as commensals in a wide range of wild and domestic birds and mammals,

including poultry, dairy cows, and domestic pets. Sources of human *Campylobacter* infection therefore are animals and animal products, especially raw milk and poultry meat, as well as untreated water, which is frequently contaminated by birds or farm animals. Water, milk, and poultry have also been involved in community outbreaks. The infectious dose is 500 organisms in milk. *C. jejuni* is an important cause of traveler's diarrhea⁸.

METABOLISM AND PHYSIOLOGY

Most *Campylobacter* species produce catalase and, apart from *C. jejuni* subspecies *doylei*, reduce nitrate to nitrite. *Campylobacter jejuni* is the only species to hydrolyze sodium hippurate. *Campylobacter* obtain their energy from amino acids or tricarboxylic acid cycle intermediaries and do not utilize sugars or produce indole. With the exception of *Campylobacter gracilis* (formerly *Bacteroides gracilis*), *Campylobacter* species are oxidase positive. Some species of *Campylobacter* can grow anaerobically in the presence of certain electron acceptors such as fumarate, aspartate or nitrate. *Campylobacter sputorum* has been described as an aerotolerant anaerobe, whilst *Campylobacter sputorum*, *Campylobacter concisus*, *Campylobacter mucosalis*, *Campylobacter curvus*, *Campylobacter rectus* and *Campylobacter hyointestinalis* usually require hydrogen for primary growth¹⁰.

SOURCES AND ROUTES OF TRANSMISSION OF CAMPYLOBACTER SPECIES TO HUMANS.

The principal reservoir of *Campylobacter* spp. is the alimentary tract of wild and domesticated birds and mammals. *Campylobacter* species are generally commensal organisms; however, occasionally they serve as enteric pathogens in the young of some species, e.g. calves, lambs and puppies. They have also been isolated from sea water, streams, rivers and estuaries which have been subjected to faecal contamination⁴.

Campylobacter species may be transmitted to humans either directly or indirectly. Direct transmission can occur via contact with infected animals, infected carcasses or infected water. Indirect transmission can occur through the ingestion of contaminated food or water¹.

Campylobacter species are widely distributed in most warm-blooded animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep and ostriches; and in pets, including cats and dogs. The bacteria have also been found in shellfish³.

The main route of transmission is generally believed to be foodborne, via undercooked meat and meat products, as well as raw or contaminated milk. Contaminated water or ice is also a source of infection. A proportion of cases occur following contact with contaminated water during recreational activities³.

Campylobacteriosis is a zoonosis, a disease transmitted to humans from animals or animal products. Most often, carcasses or meat are contaminated by *Campylobacter* from faeces during slaughtering. In animals, *Campylobacter* seldom causes disease².

The relative contribution of each of the above sources to the overall burden of disease is unclear but consumption of undercooked contaminated poultry is believed to be a major contributor. Since common-source outbreaks account for a rather small proportion of cases, the vast majority of reports refer to sporadic cases, with no easily discernible pattern¹.

Estimating the importance of all known sources is therefore extremely difficult. In addition, the wide occurrence of *Campylobacter* also hinders the development of control strategies throughout the food chain. However, in countries where specific strategies have been put in place to reduce the prevalence of *Campylobacter* in live poultry, a similar reduction in human cases is observed⁵.

Campylobacter causes gastrointestinal infection and diarrheal illness. *Campylobacter* is a zoonotic pathogen and can contaminate foods such as chickens, cattle, sheep, and pigs. On Gram stain organisms appear as seagull, shaped Gram-negative rods. Colonies will grow on *Campylobacter* selective medium such as Campy-CVA (cefoperazone, vancomycin, and amphotericin) or Skirrow media and require extra concentrations of CO₂ for growth and a temperature of 42°C. *Campylobacter* species are oxidase positive. *Campylobacter jejuni* can be differentiated from *Campylobacter coli* by hydrolysis of hippurate. Infection can be treated with azithromycin and erythromycin¹.

CAMPYLOBACTER SPECIES AND FOOD

Campylobacter species are frequently isolated from foods of animal origin. Poultry is regarded as one of the most important reservoirs for *Campylobacter* spp. and constitutes a very significant vehicle for its transmission to humans. The result of an EU wide baseline study revealed an Irish prevalence in broiler batches of 83.1% and a prevalence of 98.3% on carcasses at the end of slaughtering process. Cross-contamination of ready-to-eat foods, direct hand-to-mouth transfer during food preparation and to a lesser extent consumption of undercooked poultry meat has all been identified as important modes of transmission. It is estimated that the handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis in European Member States. Other foods associated with *Campylobacter* spp. include raw drinking milk (which may become contaminated through faecal contamination or mastitic infection), contaminated drinking water, fresh produce and /bivalve molluscs¹⁴.

Most *Campylobacter* species are zoonotic and many food animals carry the bacterium asymptomatically. Poultry and poultry products are the main means of *Campylobacter* transmission to humans. Other foods such as red meat, unpasteurised raw milk, fresh produce and contaminated water may also harbour the pathogen¹³.

Due to the specific atmospheric and temperature requirement of *Campylobacter* species, they are not capable of growing and multiplying in foods. However they have a low infective dose (of about 500-1000 cells) and a high pathogenicity meaning that only a few cells are sufficient to cause disease⁷.

Microbiological standards for *Campylobacter* specify that the bacterium should not be present in a 25g sample of ready-to-eat foods.

RISK FACTORS ASSOCIATED WITH CAMPYLOBACTER

Intake of contaminated food and water has been found in previous studies to be a major risk factor for acquisition of a *Campylobacter* infection (10). Among food-borne outbreaks, consumption of unpasteurized milk and cross-contamination events in the kitchen from raw poultry were most commonly implicated⁶.

Campylobacter species are widely distributed in most warm-blooded animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep and ostriches; and in pets, including cats and dogs. The bacteria have also been found in shellfish.

Campylobacter has a low infective dose (less than 500), which means that coming into contact with a few bacteria can cause illness. Patients infected with *Campylobacter* may experience mild to severe illness. Symptoms may include (bloody) diarrhoea, abdominal pain, fever, headache and nausea. The mean duration of illness is 2–5 days but can be up to 10 days. Infection can be associated with serious complications¹⁰.

The most common are *C. jejuni* and *C. coli* and are responsible for gastroenteritis in humans. The other emerging species such as *C. consicus*, *C. upsaliensis*, *C. ureolyticus*, *C. hyointestinalis* and *C. sputorum* have been associated with gastroenteritis and periodontitis. In some cases, infection of the gastrointestinal tract by these bacteria can progress to life-threatening extra-gastrointestinal diseases⁴.

C. jejuni is also considered as one of the most common risk factors for developing Guillain-Barré syndrome (GBS), a rare autoimmune disorder in which a person's own immune system damages the nerves, causing muscle weakness and sometimes paralysis and even fatality. About 1 in every 1,000 reported *Campylobacter* illnesses leads to GBS and its symptoms may last for a few weeks to several years. *Campylobacter* infection leads up to 40% of GBS cases in the United States⁴.

It is often difficult to trace the sources of exposure to *Campylobacter*; this is due to the sporadic nature of the infection and the important role of cross-contamination. *Campylobacteriosis* remains a problematic disease to prevent and infection epidemiological trend continues to remain high throughout the world⁹.

CAUSES OF CAMPYLOBACTERIOSIS

Campylobacteriosis is an infection caused by bacteria of the genus *Campylobacter*. These bacteria live in the intestines of healthy birds, and raw poultry meat commonly has *Campylobacter* on it¹⁵.

Campylobacter is one of the most common bacterial causes of diarrheal illness in the United States and is the most commonly reported bacterial enteric pathogen in Minnesota. Approximately 800 to 1200 cases of *Campylobacter* are reported in Minnesota each year. Virtually all cases occur as isolated, sporadic events, not as part of large outbreaks¹³.

Most *Campylobacter* infections are probably acquired by eating raw or undercooked poultry or eating something that touched it. *Campylobacter* are also transmitted by other foods, including seafood, meat, and produce; by contact with animals; and by drinking untreated water¹¹.

SYMPTOMS OF CAMPYLOBACTERIOSIS

Symptoms include:

1. Diarrhea
2. Abdominal pain and cramps
3. Fever
4. Vomiting.

Hence, symptoms usually begin within 2 to 5 days after exposure to the organism¹².

CLINICAL MANIFESTATIONS

Campylobacter species have been implicated as a predisposing factor for a range of clinical manifestations. *C. jejuni* infection leads to chronic sequelae known as Guillain–Barré syndrome (GBS) and Miller–Fisher syndrome, autoimmune conditions caused by the production of antibodies to *C. jejuni* lipooligosaccharide (LOS), which react with peripheral neural tissues resulting in acute polyneuropathy. A meta-analysis conducted by Sejvar and colleagues showed that the incidence of GBS in North America and Europe ranged from 0.81 to 1.89 (median, 1.11) cases per 100 000 person-years, with the incidence increasing by 20% for every 10-year increase in age. In populations other than Western countries, the incidence of GBS in Harbin, China, has been reported to be 0.66 per 100 000 person-years, with the highest incidence occurring in the youngest age group, in contrast to findings from Western countries⁴.

Clark and colleagues have reported that patients with acute gastroenteritis resulting from consumption of drinking water contaminated with *Campylobacter* or *E. coli* O157:H7 were at an increased risk of hypertension, renal impairment, and self-reported cardiovascular disease. *Campylobacter* species have also been reported in bacteraemia, colorectal cancer, Barrett oesophagus, lung infections, brain abscess, meningitis and reactive arthritis. A survey of the literature revealed that at least ten different *Campylobacter* species have been reported in patients with bacteraemia, with the most commonly reported being *C. jejuni* and *C. fetus*. The elderly or those who are immunocompromised are the most susceptible to *Campylobacter* bacteraemia. Bacteraemia and systemic organ and tissue infections associated with *Campylobacter* species are considered uncommon. However, it has been argued that these species are often under-reported⁵.

There is increasing evidence to suggest that *Campylobacter* species play a role in gastrointestinal conditions other than gastroenteritis and IBD. For example, Wu and colleagues performed pyrosequencing on faecal samples of 19 patients with colorectal cancer and 20 healthy controls and found that members of the *Campylobacter* genus were one of 16 genera that were significantly increased in number in patients as compared with controls. However, in their study the specific species that correlated with the increase was not determined. In another study, Macfarlane and colleagues found 57% (4/7) of patients with Barrett oesophagus to be colonized with *C. concisus* and *C. rectus* (with *C. concisus* being recovered in high numbers), but not in any of the seven healthy controls. Further in a study based on a larger cohort of subjects, Blackett and colleagues, using real-time PCR, detected members of the *Campylobacter* genus in oesophageal biopsies of 51.4% (19/37) of patients with gastro-oesophageal reflux disease and 42.2% (19/45) of patients with Barrett oesophagus, which was shown to be higher than that in patients with oesophageal adenocarcinoma (8.8%; 3/34) or controls with no endoscopic evidence of oesophageal, gastric or duodenal disease (12.8%; 5/39). Interestingly, sequencing analyses revealed all positive *Campylobacter* species to be *C. concisus*. Given that an increasing number of studies have identified *C. concisus* in the upper gastrointestinal tract, it is plausible that *C. concisus* may play a role in diseases at these sites. Consistent with this hypothesis, von Rosenvinge and colleagues found that *C. concisus* made up more than 90% of the *Campylobacter* species detected in the stomach of patients with a range of clinical manifestations, including erythematous gastropathy, gastric ulcers and Barrett oesophagus. In

addition, their study showed the levels of *Campylobacter* RNA to be increased by 444% when compared to *Campylobacter* DNA, indicating that these species were transcriptionally active in the stomach fluid. These results may suggest that *Campylobacter* species, particularly *C. concisus*, are natural colonizers of gastric tissues and could well be opportunistic pathogens in the stomach. Of particular relevance to this hypothesis is that children with CD infected with *C. concisus* generally have L4 involvement (upper gastrointestinal tract (GIT): oesophagus and stomach) [61]. Furthermore, it has been reported that children with CD and UC have an increased prevalence of *Helicobacter pylori*-negative chronic active gastritis and duodenitis than children without IBD. Given the findings from these recent studies, further studies investigating the role of *Campylobacter* species in upper gastrointestinal conditions are warranted⁷.

Campylobacter invasion antigen B (CiaB) is the first secreted factor identified in *Campylobacter* and shares weak homology to T3SS effectors of other pathogens. The CiaB protein is translocated into the cytoplasm of host cells, suggesting that it is a true effector molecule facilitating invasion. CiaB expression is essential for the secretion of a whole family of other secreted Cia proteins that are induced in the presence of FCS. However, there is no significant reduction in invasion by a *ciaB* mutant in the model strain 81-176, suggesting that further work is required to confirm the role played by this protein during infection⁶.

Campylobacter infection continues to be a major public health problem. *Campylobacter jejuni* and *Campylobacter coli* are pathogens transmitted commonly through food, causing an estimated 1.3 million cases of illness per year in the United States, and yet diagnosis can be challenging because the organism is difficult to isolate, grow, and identify. Recent reports describing clinical laboratory practices for *Campylobacter* diagnostics in Pennsylvania and the Foodborne Diseases Active Surveillance Network (FoodNet) sites highlight the wide range of testing practices in use; currently, no best-practice clinical or public health laboratory guidelines exist for laboratory diagnosis of *Campylobacter* infections. Direct plating onto a *Campylobacter* selective medium, followed by incubation at 42°C under microaerobic conditions for 72 h, has long been considered the “gold standard” for diagnosis³.

The use of culture-independent diagnostic tests (CIDTs) for *Campylobacter* testing on stool samples is increasing, which may have important implications for both patient management and public health surveillance efforts. Stool antigen tests to directly detect *Campylobacter* in fecal samples are fast and generate same-day results, but concerns regarding specificity and positive predictive value (PPV) have been raised. There are currently no guidelines on how to interpret and report discordant results between stool antigen tests and culture. In addition, the current national case definition for a confirmed case of *Campylobacter* requires culture confirmation, whereas persons with positive CIDTs only are classified as probable cases. Current reports of *Campylobacter* incidence and trends through FoodNet are also based only on culture-confirmed cases, though CIDT results are tracked. There is a clear need to evaluate the performance of culture-independent assays, to better inform microbiologists and clinicians on the use of these tests in patient management, and to ensure the validity of public health surveillance data and also for real-world data for informed considerations on whether and how *Campylobacter* case definitions should be modified. To better understand the performance characteristics of stool antigen CIDTs for *Campylobacter* diagnosis, we conducted a prospective, multicenter study to evaluate the performance of stool antigen tests in comparison to culture and PCR for detection of *Campylobacter* from stool¹.

CLINICAL DIAGNOSIS/LABORATORY APPROACH IN NIGERIA

Methods for Identification and Detection of *Campylobacter*:

Campylobacter infection is diagnosed when a laboratory test detects *Campylobacter* bacteria in stool (poop), body tissue, or fluids. The test could be a culture that isolates the bacteria or a rapid diagnostic test that detects genetic material of the bacteria⁷.

Stool Antigen Tests.

The following four stool antigen tests can perform on all stool specimens at each study site according to the manufacturer's instructions. Two are formatted as microplate assays, ProSpecT *Campylobacter* (Remel Inc., Lenexa, KS) and Premier *Campy* (Meridian Bioscience Inc., Cincinnati, OH), and two are formatted as lateral flow devices, ImmunoCard Stat! *Campy* (ICS; Meridian Bioscience Inc., Cincinnati, OH) and Xpect *Campy* (Remel Inc., Lenexa, KS).

Polymerase Chain Reaction (PCR).

For molecular diagnosis, genomic DNA can be isolated using either the QIAamp DNA stool minikit or the automated QIAcube system (Qiagen, Valencia, CA) according to the manufacturer's instructions. Study sites tested all stool specimens using the Seeplex diarrhea-bacterial panel 1ACE detection PCR kit (Seegene Inc., Seoul, South Korea) according to the manufacturer's instructions. Four study sites (CO, GA, MN, and PA) performed PCR testing for their own respective specimens, and study site 4 (GA) performed PCR testing for the other four sites (CA, CT, IA, and MD). Stool specimens were shipped frozen on dry ice, to study site 4 for this PCR testing, once all other testing was complete. This multiplex PCR kit is based on dual priming oligonucleotide technology (DPO), which detects *Campylobacter jejuni* and *Campylobacter coli* (but does not differentiate between these two *Campylobacter* species), *Salmonella* spp. (*Salmonella bongori* and *Salmonella enterica*), *Shigella* spp. (*Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, and *Shigella dysenteriae*), *Vibrio* spp. (*Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*), and *Clostridium difficile* toxin B. This PCR assay has been previously validated and is reported to be more sensitive than culture for detection of *Campylobacter* from stool³.

Culture-Based Methods

Culture-based techniques for isolation and detection of *Campylobacter* from foods are described by the guidelines of International Standards Organization. ISO 10272-1:20063 defines the procedures for detection while ISO/TS 10272-2:20064 specifies the procedure for enumeration⁴.

The pathogen is isolated by culturing on selective media followed by incubation at 41.5°C for 44 hours under microaerobic conditions. Food and environmental samples need an additional pre-enrichment step designed to facilitate the recovery of damaged cells. Enrichment is done using a selective enrichment broth medium which is then incubated at 37°C for five hours. Clinical samples can be cultured directly onto selective media¹⁴.

Following isolation, *Campylobacter* identification is carried out based on their morphological, biochemical and growth characteristics. Most used identification schemes include Gram staining and biochemical tests such as catalase, oxidase, hippurate hydrolysis, and nitrate/nitrite reduction¹³.

Several selective agars designed to isolate *Campylobacter* colonies are commercially available. These media contain various selective agents, most of which are antibiotics that suppress the growth of other enteric bacteria. Pre-enrichment media contain ingredients that protect the cells from the damaging effects of toxic oxygen derivatives. These include lysed or defibrinated blood; charcoal; a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP)⁵.

Both selective and enrichment media can be purchased as either base powders or ready-to-use formulations. In addition, commercially available atmosphere generation systems can be used to provide the specific microaerobic atmosphere which is critical for *Campylobacter* recovery and enumeration⁷.

Although these culture-based methods are relatively cost effective and require no sophisticated equipment, they have several limitations. Most significant drawbacks include the time required to obtain the final results and the limited response of *Campylobacter* to biochemical tests. Moreover, these techniques are labour intensive and have lower sensitivity compared to serological and molecular methods. There is also the possibility of *Campylobacter* cells entering the viable but not culturable (VBNC) state under unfavourable conditions, thus providing false negative results.

Various methods of culture preparation can be done to accelerate the enrichment process. These methods include cell separation and concentration by filtration or centrifugation.



Fig 8 :



fig 9 :

Comment [HS4]: Add photo source

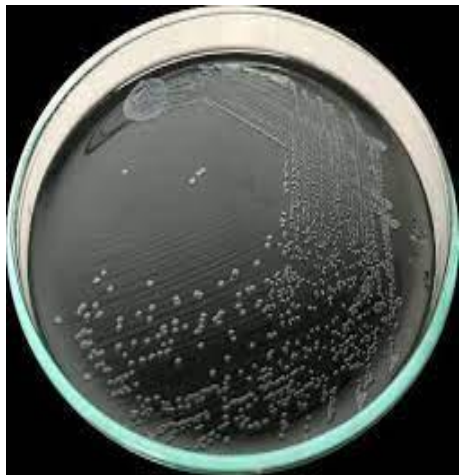


Fig 10 :



fig 11 :

Fig. 8-11. Various methods of culture preparation

(Jeanette *et al.*, 2006)

Rapid detection methods

Several immunological and molecular techniques are commercially available for the detection and identification of *Campylobacter*. These techniques offer rapid, accurate and more sensitive results compared to the traditional methods. Another advantage of these methods is that these techniques can detect *Campylobacter* cells in the VBNC state. However, some of these methods demand more advanced instruments as well as specially trained individuals. They also fail to distinguish between dead and live cells⁸.

Various immunoassay systems based on antibody/antigen interactions such as enzyme-linked immunosorbent assay (ELISA) and Latex agglutination are commercially available.

Nucleic acid-based methods such as polymerase chain reaction (PCR) and real-time PCR are also commonly available as commercial kits. Other molecular techniques including Pulsed Field gel Electrophoresis (PFGE) and Random Amplified Polymorphic DNA (RAPD) can also be applied for detection and identification of the organism⁹.

Furthermore, a combination of traditional and modern techniques can be used to further enhance the reliability and speed of the result¹⁴.

TREATMENT

Treatment is not generally required, except electrolyte replacement and rehydration. Antimicrobial treatment is recommended in invasive cases (when bacteria invade the intestinal mucosa cells and damage the tissues) or to eliminate the carrier state (the condition of people who harbour *Campylobacter* in their bodies and keep shedding the bacteria while remaining asymptomatic)⁶.

Most people recover from *Campylobacter* infection without antibiotic treatment. Patients should drink extra fluids as long as diarrhea lasts.

Some people with, or at risk for, severe illness might need antibiotic treatment. These people include those who are 65 years or older, pregnant women, and people with weakened immune systems, such as those with a blood disorder, with AIDS, or receiving chemotherapy¹².

Some types of antibiotics may not work for some types of *Campylobacter*. When antibiotics are necessary, healthcare providers can use laboratory tests to help determine which type of antibiotics will likely be effective¹⁵.

People who are prescribed antibiotics should take them exactly as directed and tell their healthcare provider if they do not feel better¹¹.

PREVENTION

There are a number of strategies that can be used to prevent disease from *Campylobacter*:

Prevention is based on control measures at all stages of the food chain, from agricultural production on a farm, to processing, manufacturing and preparation of foods both commercially and domestically¹⁰.

In countries without adequate sewage disposal systems, faeces and articles soiled with faeces may need to be disinfected before disposal.

Measures to reduce the prevalence of *Campylobacter* in poultry include enhanced biosecurity to avoid transmission of *Campylobacter* from the environment to the flock of birds on the farm. This control option is feasible only where birds are kept in closed housing conditions¹.

Good hygienic slaughtering practices reduce the contamination of carcasses by faeces, but will not guarantee the absence of *Campylobacter* from meat and meat products. Training in hygienic food handling for abattoir workers and raw meat producers is essential to keep contamination to a minimum².

Prevention methods against infection in domestic kitchens are similar to those used against other foodborne bacterial diseases.

Bactericidal treatment, such as heating (for example, cooking or pasteurization) or irradiation, is the only effective method of eliminating *Campylobacter* from contaminated foods³.

WORLD HEALTH ORGANIZATION (WHO) RESPONSE

In partnership with other stakeholders, WHO is strongly advocating the importance of food safety as an essential element in ensuring access to safe and nutritious diets. WHO is providing

policies and recommendations that cover the entire food chain from production to consumption, making use of different types of expertise across different sectors⁷.

WHO is working towards the strengthening of food safety systems in an increasingly globalized world. Setting international food safety standards, enhancing disease surveillance, educating consumers and training food handlers in safe food handling are amongst the most critical interventions in the prevention of foodborne illnesses⁹.

WHO is strengthening the capacities of national and regional laboratories in the surveillance of foodborne pathogens, such as *Campylobacter* and *Salmonella*.

WHO is also promoting the integrated surveillance of antimicrobial resistance of pathogens in the food chain, collecting samples from humans, food and animals and analyzing data across the sectors¹³.

WHO, jointly with FAO, is assisting Member States by coordinating international efforts for early detection and response to foodborne disease outbreaks through the network of national authorities in Member States⁵.

WHO also provides scientific assessments as basis for international food standards, guidelines and recommendations developed by the FAO/WHO Codex Alimentarius Commission to prevent foodborne diseases¹.

RECOMMENDATIONS FOR THE PUBLIC AND TRAVELLERS

The following guidance will help people to stay safe while travelling:

Ensure food is properly cooked and still hot when served.

Avoid raw milk and products made from raw milk. Drink only pasteurized or boiled milk.

Avoid ice unless it is made from safe water.

When the safety of drinking water is questionable, boil it, or if this is not possible, disinfect it with a reliable, slow-release disinfectant agent (usually available at pharmacies).

Wash hands thoroughly and frequently using soap, in particular after contact with pets or farm animals, or after having been to the toilet.

Wash fruits and vegetables carefully, particularly if they are eaten raw. If possible, vegetables and fruits should be peeled.

RECOMMENDATIONS FOR FOOD HANDLERS

WHO provides the following guidance for people handling food:

Both professional and domestic food handlers should be vigilant while preparing food and should observe hygienic rules of food preparation.

Professional food handlers who suffer from fever, diarrhoea, vomiting, or visible infected skin lesions should report to their employer immediately.

The WHO *Five keys to safer food* serve as the basis for educational programmes to train food handlers and educate consumers. They are especially important in preventing food poisoning. The Five keys are:

1. Keep clean
2. Separate raw and cooked
3. Cook thoroughly
4. Keep food at safe temperatures
5. Use safe water and raw materials.

CONCLUSION

Campylobacter is a bacterial species that represent one of the most common causes of diarrheal illness worldwide. This infection is associated with the consumption of undercooked poultry, raw milk, and contaminated water. Patients typically present with a self-limited diarrheal illness lasting 5 to 7 days.

Despite campylobacteriosis being the most important bacterial foodborne disease in the developed world there is limited success in strategies to combat this disease. Herein we highlight a so far underestimated perspective of this pathogen where it takes advantage of other microorganisms.

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