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REVIEW ARTICLE

Campylobacter Species in Poultry: Virulence Attributes, Pathogenesis, Epidemiological Typing and Zoonotic Importance

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Abstract

Campylobacter species (spp.) are Gram-negative, curved, S-shaped, non-spore forming and motile rods with a single polar flagellum. They represent the most common causes of human foodborne gastroenteritis. Campylobacter colonizes the gastrointestinal tract (GIT) of a wide variety of domestic and wild animals, particularly chickens, turkeys and pigs, which are considered the main reservoirs of this bacterium. Campylobacter is transmitted to human, mainly through ingestion of contaminated poultry meat, unpasteurized milk and polluted water, causing severe abdominal pain, fever, fatigue and diarrhea. Nevertheless, little knowledge about the biology and pathogenicity of *Campylobacter* spp. is known rather than other predominant pathogens. Therefore, we reviewed the biology of the bacterium, its survival, growth characters and the factors related to its pathogenicity and the mechanisms by which the diseases are happened in the view of the available literatures. Furthermore, we illustrated several techniques used for *Campylobacter* spp. epidemiological classification.

Keywords: *B. cereus*; biofilm; dairy products; *Nigella sativa*; olive oil's nanoemulsions.

Introduction

Thermotolerant *Campylobacter* spp., particularly *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* ranked the second most emergent bacteria after *Salmonella* infection in its zoonotic importance [1, 2]. Campylobacteriosis is a self-limiting disease with typical gastroenteritis symptoms that only last few days. However, some drawbacks like reactive arthritis or Guillain-barre syndrome (GBS) have been rarely associated with campylobacteriosis [3].

Campylobacter colonizes the gastrointestinal tract (GIT) of a wide host range including chickens, turkeys and pigs, which are the microorganism's principal reservoirs [4]. Human gut infected by handling and/or consumption of improbably prepared chicken meals, unpasteurized milk and contaminated water [5, 6].

Campylobacter established infection because of numerous virulence characteristics such as motility, intestinal adherence, colonization, toxin syntheses and invasion. Mobility of the bacterial cells, involving the coordination of several genes (i.e., *flaA* and *flhA*), is essential for passage through the stomach and gut environment [7], where Campylobacter produces several cell-surface proteins (encoded by *cadF*, *docA*, *racR*, *virB11*, *ciaB*, and *iam* genes) that promote adhesion to and invasion of intestinal epithelial cells [8, 9]. The bacteria can also produce cytotoxins that contribute to the development of the disease [10, 11].The cytolethal distending toxin (CDT) is one of the most well-studied toxin that causes death in the host epithelial cells [10, 12]. The CDT gene cluster consists mainly of three subunits; *cdtA*, *cdtB* and *cdtC* [13].

Campylobacter populations comprise several genotypes with a significant surface

antigen modification; capsule, lipooligosaccharides (LOS) [14] and flagella [15]. This great difference in Campylobacter populations doesn't enable us to understand the disease's epidemiology even to identify the genus or species levels of Campylobacter [16]. Molecular techniques are applied for typing of foodborne bacterial pathogens, characterizing the intra-species variability of an organism and tracking the strains with similar or identical fingerprinting patterns in epidemiological studies [17].

Owing to the important role of molecular typing techniques in understanding the epidemiology and the global dramatic increasing in the drug resistant *Campylobacter* spp., there are urgent needs to investigate the continuous variation and survey outbreaks and to track the source of these microorganisms [18-20]. Herein, we reviewed the survivability, growth characters, pathogenicity factors and epidemiological typing for *Campylobacter* spp.

General characters of *Campylobacter* species

Campylobacter spp. are Gram-negative bacteria that do not produce spores [21]. They are S-shaped or spiral rods with a width of 0.2-0.9 µm and a length of 0.5-5 µm. Changes from spiral to coccoid form occurred in prolonged exposure to air and/or old cultures [22]. Most *Campylobacter* spp. are motile with the bacteria rotating around their longitudinal axis by a single unsheathed polar flagellum with monotrichate or amphitrichate arrangements [23]. The only exceptions are C. *showae*, which has up to five unipolar flagella and C. *gracilis*, which is none motile [21]. *Campylobacter* spp. are easily pass through bacterial filters (0.45 to 0.65 µm) due to their characteristic mobility and tiny size; this property is utilized to isolate *Campylobacter* spp. from clinical samples [24].

Campylobacters show obvious growth after 24 - 48 hours at 37 °C under perfect conditions; nevertheless, some slow-growing strains may take up to 72-96 hours to be detected [25]. Depending on the media employed, the morphology of Campylobacter colonies may change. When the media is moist, the colonies may seem grey, flat, uneven and thinly spreading; while when the media is dry, the colonies may appear round, convex or shiny [23]. The optimal growth temperature of thermophilic *Campylobacter* spp. is 41.5 °C; however, because they do not grow at 55°C or higher, the term "thermotolerant" is more appropriate than thermophilic [26]. Campylobacters are unable to adapt or grow at a temperature below 30°C due to a lack of cold shock genes [2].

These fastidious non-spore forming bacteria acquire power from the breakdown of amino acids or tricarboxylic acid cycle byproducts and they neither oxidize nor ferment carbohydrates [27, 28].The most suitable for Campylobacter's incubation is microaerophilic conditions with a little oxygen pressure [29].

Campylobacter spp. are very sensitive to numerous environmental changes; for instance temperature and moisture, oxygen level, pH changes, ultra violet irradiation and disinfectants [30]. They inactivated by heating with a D-value (decimal reduction time) of less than one minute [2]. Despite the fact that freezing and thawing reduce viable populations by 1-2 $\log_{10}$, the bacteria can survive for months at -20 °C [23]. The ideal growth of *Campylobacter* spp. occurs at pH 6.5-7.5 [2]. Viable but non-cultivable (VBNC) cells state of *C. jejuni* and *C. lari* is occurred due to exposure to unfavorable conditions [31], indicating that they can't grow during the subculture [31, 32].

Cytolethal distending toxins in *Campylobacter* species

Campylobacter spp. established the infection because of numerous virulence characters including motility, intestinal adherence, colonization, toxin syntheses and invasion. Adherence of the microorganism to epithelium cells of the intestine is vital for colonization and toxins production [33, 34].

CadF is a vital preserved cell surface protein in *C. jejuni* and *C. coli* that binds to intestinal fibronectin aiding in adherence and invasion [33, 34].This bacterium is motile by flagella to reach the adherence receptors in the intestine [35]. Heat shock proteins including DnaJ have been related to the thermal stress response and they have a significant role in Campylobacter pathogenesis [36].

Bacterial toxins play a significant role in the pathogenesis of Campylobacter infection. The cytolethal distending toxin (CDT), the most important toxin of *Campylobacter* spp. is not restricted to these bacterial species, but it was detected in many other bacteria including *Escherichia coli* (*E. coli*), *Shigella* spp., *Helicobacte*r *hepaticus, Haemophilus ducreyi,* and *Actinobacillus actinomycete mcomitans* [37]. CDT is a bacterial protein toxin that affects the epithelial cell layer and interrupts the cell division process with resulting cell cycle arrest and cell death [38, 39]. It is composed of three subunits"AB2", in which CdtB is the active part (A unit), while CdtA and CdtC make up the "B2" units, which are essential for CdtB attachment and transportation to target cells [40]. *C. jejuni cdtA*, *cdtB*, and *cdtC* genes in *cdt* operon encoded proteins of 27, 29, and 20 kDa molecular weights, respectively [38].

Campylobacter spp. and some of enteropathogenic *E. coli* produced CDT in culture supernatants causing gradual eukaryotic cells distension and cell death within 2-5 days [39] (Figure1). The virulence properties of CDT+ and CDT− strains on HeLa cells revealed that CDT+ *C. jejuni* strains adhere to and invade epithelial cells more efficiently than CDT− strains. The DNase activity of CdtB subunit causes termination of cell division and arrests the eukaryotic cell cycle at the G2/M stage (i.e. a period of rapid cell growth and protein synthesis during which the cell prepares itself for mitosis) [40-42]. The existence of the *cdtB* gene in *C. jejuni* is associated with enhanced adhesion, invasion and cytotoxicity in HeLa cells [41].

The prevalence of *cdt* genes was detected by PCR in all campylobacters except one *C. jejuni* isolate obtained from Danish broilers [43], while more than 80% of the tested *C. jejuni* strains encoded *cdt* genes in Bahrain [44]. Moreover, *cdt* genes were present in all Campylobacter strains including *C. jejuni* and *C. coli*, those were recovered from chicken feces in Southern Iran [45]. However in Egypt, the prevalence of *cdt* genes in *C. jejuni* isolated from avian and human sources, were estimated in a recent study and the results indicate that *cdtA*, *cdtB*, and *cdtC* were detected in the analyzed strains by the same percentage (80.49%), and more than half (58.54%) of the strains possessed the three *cdt* toxin genes together [46].

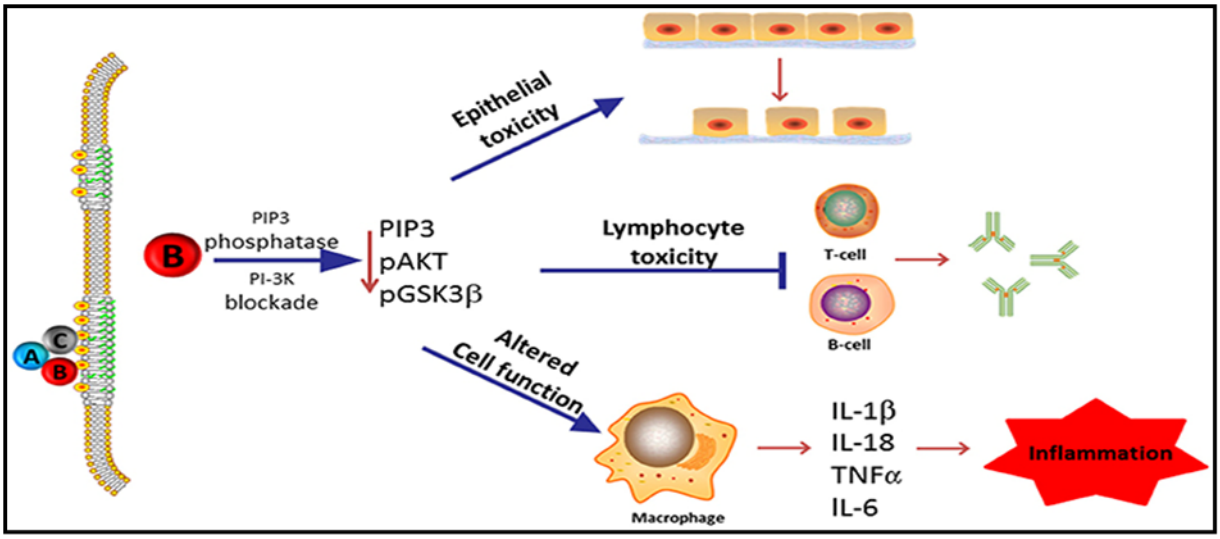

Figure 1: An overview of CDT toxin impairing host defense. CDT toxin have three ways to impair the host defense mechanism; (1) Disrupt epithelial barriers and facilitate pathogen infection by induction of apoptosis, (2) Promote lymphocyte cytotoxicity, disrupt acquired immunity and promote persistent infection and (3) Increase cytokine synthesis resulting in a pro-inflammatory response that alternates the macrophage functions; retrieved from Scuron and coauthors [47].

Pathogenesis of enteric Campylobacter infection

Until now, the pathogenesis of Campylobacter infection is not fully understood; however, it is thought that motility, colonization, invasion and toxin production have a vital role in the establishment of infection [9, 48]. The mucus layer of the gastrointestinal tract (GIT) epithelium acts as the first line of defense, but numerous traits due to the ability of *C. jejuni* to penetrate and evade the mechanical and immunological barriers of the GIT are responsible for establishment of an infection. The motility encoded by several genes; i.e., *flaA* and *flhA* [7], corkscrew morphology and the relatively short O-sidechain of *C. jejuni* LOS are thought to decrease the non-specific binding to the mucin glycoproteins [49]. Furthermore, numerous cell-surface proteins synthesized by campylobacters help in early colonization, adhesion and invasion of intestinal epithelial cells [8, 9, 50]. Various cytotoxins encoded by the *cdt* gene locus and *wlaN* gene are contributed to established diseases [10]. Moreover, one of the most important bacteria's key defenses against oxidative harm is its ability to get rid of the effect of superoxide radicals via the production of superoxide dismutase enzyme [51]. While bacterial virulence factors make allowance for survival of the bacteria within host cells, the resultant immune response is responsible for the clinical manifestations of infection [9, 52]. For example, CDT production promotes immune system evasion, and simultaneously activates the inflammatory response through interleukin 8 (IL-8) stimulation [53] and toll-like receptors activation on GIT epithelial cells and dendritic cells. This stimulates both the innate and adaptive immune pathways, allowing the mobilization of inflammatory cells that are responsible for the resultant diarrhea, as well as the ultimate clearance of the organism [54, 55]. *C. jejuni's* interaction with the intestinal epithelial cells leads to an arrest in the proliferation of cells at the crypts with a consequence of villous atrophy [33] (Figure 2).

Campylobacter infection may cause various persistent diseases including GBS, irritable bowel syndrome, Miller Fisher syndrome and Reiter's arthritis in humans. The structural similarity between human neuronal gangliosides and Campylobacter LOS leads to cross-reactivity or non-specific binding of anti-LOS antibodies with human neuronal gangliosides causing GBS [56, 57].

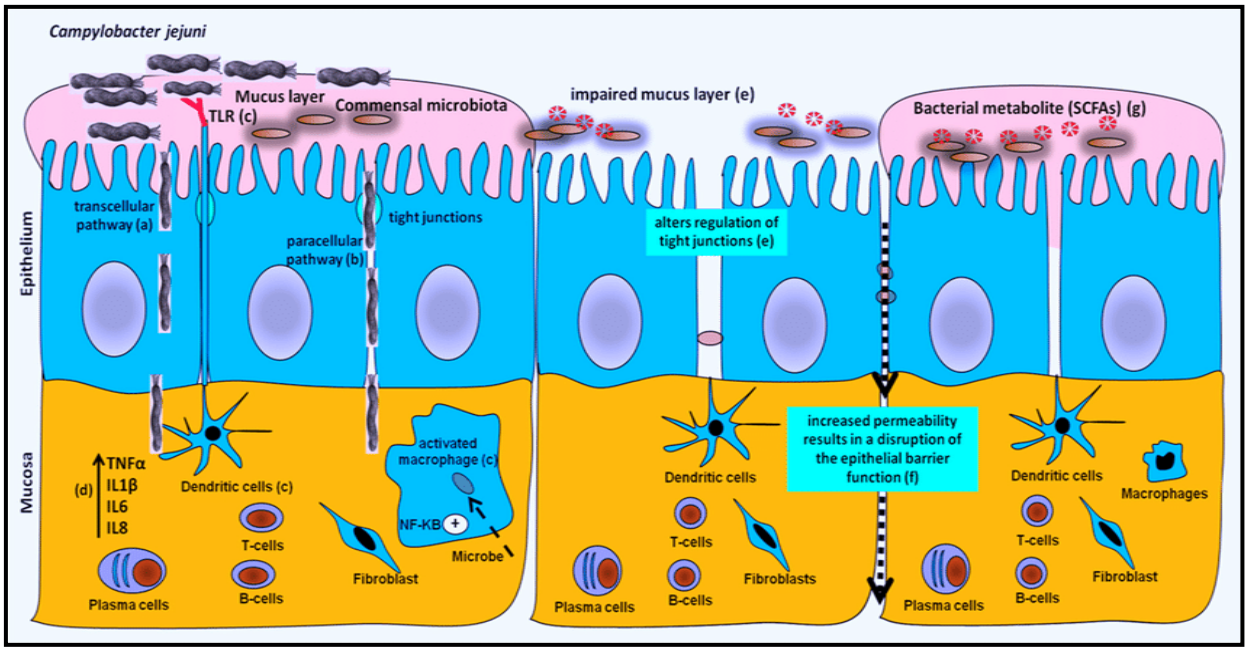

Figure 2: Campylobacters disordered GIT physiological processes in the host. Translocation occurs through transcellular (a) and paracellular pathways (b). Toll like receptors of pathogenic microorganisms are recognized by macrophages and dendritic cells (c) causing change in their functional status to an activated form. The activation of nuclear factor-kB (NF-kB) pathway stimulates gene transcription, resulting in increased production of pro-inflammatory cytokines (TNF-α, interleukins 1β, IL 6 and IL8) (d). Moreover, campylobacters induce a disruption of tight junctions and the mucus film (e) leading to passage of luminal antigens (e.g., microorganisms and toxins) as a result of increased intestinal epithelial permeability (f). Furthermore, campylobacters utilize short chain fatty acids (SCFAs) as a source of energy leading to changes of gut colonization dynamics and may also influence physiological processes due to altered microbial metabolite profiles (g); retrieved from Awad and coauthors [58].

Natural habitat of *Campylobacter* species

Campylobacter infection in poultry

The most common habitat for *Campylobacter* spp. are poultry and other avian species, owing to an elevated body temperature, representing the chief source of infection for humans [2]**.** While *C. jejuni, C. coli* and *C. lari* are related to the poultry digestive system and also to foodborne infections, *C. jejuni* is considered the most dominant species in relation to its impact on human health [59, 60].

The predominant site for *C. jejuni* colonization is avian ceca with 10^6 to 10^8 colony forming unit (CFU)/g [61]. Successful *Campylobacter* spp. colonization of chickens requires only ingestion of 35 CFU [62], followed by an established infection within 24 hours after the bacterial entry [63]. The susceptible age is between two to four weeks with no evidence of young chicks infection that may be due to the existence of maternally generated antibodies [64-66]. The successful and persistent *Campylobacter* spp. colonization of the chicken GIT occurred within several days after ingestion [67] and stayed until slaughter [63, 68] is a multi-factorial process due to the regulatory effect of several genes that converse protection against the surrounding environment [69]**.**

Factors involved in colonization of *Campylobacter* species in chickens

1. Resistance to multiple drugs and bile

Resistance to a wide range of antimicrobial agents, heavy metals and bile salts is encoded by multidrug efflux pump mediating a successful intestinal colonization of chickens [70, 71].

2. Chemotaxis

The motility of *C. jejuni* towards favorable circumstances is caused by several chemotaxis that works for its survival and colonization at the intestinal mucosa [72]**.**

3. Flagella and motility

Type III secretion system plays a role in construction of Campylobacter invasion

antigens (Cia proteins) and flagellar apparatus [35, 73] helping in reaching the mucus layer of the cecal crypts [74] and overcoming the gut peristalsis that are vital for colonization and cell invasion [75].

4. Immune evasion and carbohydrate structures on Campylobacter surface

Lipooligosaccharides are significant for immune evasion, epithelial cell adherence, penetration and invasion. *C. jejuni* is the only prokaryote known to have unique N-linked glycosylation modification pathway that is conserved among this bacterium and it is encoded by the *pgl* multigene locus. The N-linked glycosylation pathway is responsible for post-translational modification of multiple proteins, including flagellin, unique N-linked glycans, contribute to successful colonization in chicks by creating a huge antigenic diversity in *C. jejuni* isolates resulting in persistent high-level gut colonization of certain strains [76-78]**.**

5. Two-component regulatory system

Response regulators (R) and histidine kinases sensor(s) are two-component regulatory systems (TCRSs) that enable *C. jejuni* adaptation to changed environmental condition via regulating their genes expression [79, 80]. A histidine kinase detects certain environmental stimuli through autophosphorylation of the histidine residue. The phosphate group is then transferred to the corresponding response regulator, transforming it into an active transcription factor that can stimulate differential expression of target genes, allowing *C. jejuni* to respond quickly to changes in the chicken gut environment, such as stress, nutrients, and temperature [80].

6. Temperature regulation and heat shock response

Many different proteins are specially transcribed via RacR/RacS signal transduction pathway in response to higher body temperature of chicken GIT (42°C) compared to humans that play a significant role in *C. jejuni* colonization of chickens [81].

7. Adhesion

The remarkable effect of flagella, adhesins and surface-attached proteins in colonization has been showed previously [82]. Moreover, an autotransporter lipoprotein encoded by Campylobacter adhesion protein A is thought to be crucial in attachment to human and chicken intestinal epithelial cells resulting in initial colonization and invasion [83, 84].

8. Invasion

Methyl-accepting chemotaxis proteins (MCP) encoding *tlp1*, *tlp4*, and *tlp10*, and *ciaB* genes are essential for invasion through mammalian and chicken cells, respectively and they significantly influence cecal colonization [73, 85].

9. Iron transport and regulation

Iron is required for electron transfer; it acts as a catalyzer for different enzymes and produces hydroxyl radicals. Furthermore, iron availability is regulated by intracellular iron concentration and it modulated Campylobacter gene transcription helping in the success of colonization [86].

10. Oxidative and nitrosative stress defense

C. jejuni is a microaerophilic microbe that requires low oxygen levels for its growth. It has a broad range of enzymes that overcome the oxidative stress resulted from Campylobacter inadequate oxygen reduction and surrounding the host environment such as cytochrome c peroxidases that converts hydrogen peroxide to water [87]. However, Hermans and coauthors [82] previously recognized numbers of these regulators, but the exact gene regulation mechanism remains a mystery.

11. Central intermediary and energy metabolism

C. jejuni contains essential enzymes required for a tricarboxylic acid cycle. The conversion of succinate to fumarate is an important step in this cycle and that is catalyzed by fumarate reductase and a succinate dehydrogenase, which are considerably increased in the chick cecum [79, 88].

Campylobacter infection in human

Campylobacter spp. are considered one of the most common causes of foodborne related gastroenteritis around the world [89, 90] with the majority of infection caused by *C. jejuni*,

followed by *C. coli* and rarely *C. lari* [91]. Infection is occurred by direct or indirect contact with contaminated food or drinks, mainly undercooked poultry meat, untreated water and unpasteurized milk [92-94]. Campylobacteriosis is also caused by direct and indirect contact with infected poultry, animals and their wastes. The symptoms of the disease are clinically indistinguishable between species and are in the form of watery or bloody diarrhea, fatigue, fever, abdominal pain and cramps that mimic appendicitis appeared 24 to 72 hours after ingestion [95].

Campylobacter spp. cause a variety of symptoms in various regions of the body in addition to gastroenteritis. These include GBS, a neurologic disorder that causes gradual symmetrical weakness in the limbs with or without hyporeflexia and impairs respiratory and cranial nerve-innervated muscles. Miller Fisher syndrome, a similar form of GBS, is characterized by acute onset ophthalmospasms, areflexia, and ataxia [96]. Other clinical symptoms were also reported including meningitis, brain abscesses, bacteremia, sepsis, endocarditis, myocarditis and reactive arthritis [97].

Campylobacteriosis is a self-limiting disease and the symptoms ended within one week [98]. Death is rare occurred; however, sporadic death cases have been reported in young children, elderly and immunocompromised individuals [99].While campylobacteriosis can occur in all ages, infections are most commonly happened in young ages up to 24 years than in other age groups [100] due to acquiring a level of protective immunity in this older ages [101]. Furthermore, the infection with *C. coli* is commonly occurred in older patients over 30 years and traveler people overseas [102]. Generally, increased Campylobacter infections are occurred during the summer months [100].

Epidemiological analysis of *Campylobacter* species

Bacterial typing or subtyping is the way for categorizing bacterial strains to species or subspecies levels. Taxonomy assessment, phylogenetic relationships evaluation, evolution reporting and performance of rapid, precise and effective epidemiological surveillance and prevention measures are the chief goals of bacteriological typing [103]. The capability to differentiate campylobacters below the species level has been effectively used to enhance the epidemiologic research of campylobacteriosis epidemics, compare cases with possible carriers of infection, and distinguish them from unrelated strains [104-106].

Campylobacter spp. typing is a rapidly evolving field, as old methods being updated and new methodologies being developed all the time. There hasn't been a single technique considered generally suitable and appropriate [107], since each one has both advantages and disadvantages [108]. Efficacy and efficiency are two essential features that every type system should exhibit in order to be modified for further use [109] while evaluating subtyping approaches. Any typing method's efficacy can be measured in terms of reproducibility, typeability, consistency, and discrimination power; whereas efficiency indicates the required knowledge, time spent or rapidity of the technique, adaptability, and applicability for a certain investigation [110].

Campylobacter spp. typing is a dynamic field with older methods continually being advanced and new methodologies constantly being developed [107]. A multitude of typing systems have been developed over the last few years, however, no single technique has been declared as universally acceptable and applicable since each one has both advantages and disadvantages [108]. A number of criteria are used to evaluate subtyping methods to define their efficacy and efficiency: two major properties that any typing system should possess in order to be adapted for further use [109]. The efficacy of any typing technique can be assessed in terms of typability, reproducibility, consistency, and power of discrimination; while, the efficiency reflects the expertise required, time consumed or rapidity of the technique, flexibility, and suitability to carry out a certain investigation [110].

Typing systems are based on sharing common characteristics between clonally related isolates that differentiate them from unrelated isolates [111]. They are grouped into two categories: phenotyping and genotyping. Phenotyping uses phenotypic approaches to identify the presence or absence of biological or metabolic activities expressed by the bacteria, while genotyping uses genotypic approaches to analyze genetic materials based on the bacteria's DNA and RNA [112].

Phenotypic techniques used for typing of *Campylobacter* species

Biotyping, phage typing, serotyping and multilocus enzyme electrophoresis (MLEE) are the most frequently used phenotypic methods to differentiate Campylobacter isolates. Although most of these techniques lack a discriminatory power, poor reproducibility and stability, they are remaining used and are fairly effective in classifying foodborne pathogens [113]. Biotyping can classify *C. jejuni*, *C. coli* and *C. lari;* it is helpful, as a first step, for epidemiological studies based on distinguishing the bacterial isolates via colonial morphology and biochemical reactions [111, 114]. Combining biotyping with serotyping makes this approach more effective, because it is simple to be achieved, moderately cheap and can immediately detect the bacteria for additional testing [66].

Serotyping is a method of differentiating strains based on antigens carried on their surface structure using specific antisera [112]. In the 1980s, two complementary and quite well serotyping systems were designed; both give good epidemiological discrimination of Campylobacter isolates when used together. The first system is based on heat stable O antigens [Lipopolysaccharide (LPS), LOS, and capsular polysaccharide (CPS)], which are used in a passive hemagglutination method [115] . The other one is based on heat labile antigens using a bacterial agglutination method [116].

Phage typing was frequently used as an assistant to serotyping to characterize *C. jejuni* [117] with limited usefulness due to the presence of non-typeable isolates and difficulties of cross reactivity [66]. Concisely, this method uses a collection of pathogenic phages infecting a bacterial host that lacks attachment receptors. If the phages are successful in attaching to and infecting their hosts, they lyse the bacteria leaving a distinctive lytic shape on the cultivated Petri dishes known as 'plaques' [117].

By electrophoresis under non-denaturing circumstances, bacterial isolates are differentiated using a MLEE technique by differences in the electrophoretic mobility of distinct component enzymes [113] . This technique has been used to study the congruence between other typing schemes used for *C. jejuni*, such as multilocus sequence typing (MLST) and pulse field gel electrophoresis (PFGE) [105]**.**

Due to its limitations, MLEE was considered unsuitable for routine typing and was replaced by MLST, a nucleotide-based approach that essentially mirrors the MLEE's multi loci principle [110] .

Genotyping methods used for identification of *Campylobacter* species

Due to the constraints of phenotypic subtyping methods, a variety of molecular subtyping approaches have been developed [118]. Molecular techniques have become extensively used for subtyping *C. jejuni*, because they provide more sensitive strain distinction, greater degree of standardization, reproducibility and discriminatory efficiency when compared with phenotypic typing approaches [104, 111]. Molecular subtyping methods are divided into two primary types; macro-restriction mediated studies those are based on separation of restriction enzyme digested target sequences and polymerase chain reaction (PCR) based assays [110].

Pulse field gel electrophoresis (PGFE) has emerged as one of the most effective molecular methods for studying Campylobacter infections [111, 119]. Because of its excellent discriminating power, the PFGE is regarded the "golden standard" for epidemiological studies [105]. Although PFGE data is difficult to interpret making it inappropriate for routine usage during

epidemic investigations [105], it has been extensively employed in genomic and epidemiological studies of *C. jejuni* and *C. coli* [110, 119].

PCR has completely revolutionized molecular epidemiological studies because of its ability to detect an unique single gene in each organism as a result confirm its presence in any sample [110]**.** Several changes on the basic PCR method have developed including reverse-transcriptase PCR, multiplex PCR and quantitative real-time (qRT)-PCR, those are used for *Campylobacter* spp. detection [111]**.** Multiplex PCR assays are employed to differentiate *Campylobacter* spp. and they are remarkably replaced monoplex PCR assays for *Campylobacter* spp. detection and differential diagnosis [120, 121]. Although these techniques may be expensive, they are easy to replicate, discriminatory, already available and remain one of the most widely used genotypic methods for *Campylobacter* spp. typing [111]**.**

The simplicity and rapidity make PCR techniques as the frequently used genotyping and diagnostic tools [120]. The PCR-based approaches for Campylobacter genotyping include random amplified polymorphic DNA and amplified length polymorphism have high discriminatory power, but they are not widely employed due to certain limitations [110]. Ribotyping is a rRNA-based approach for identifying bacterial isolates; although having a high level of typeability for *Campylobacter* spp., they have a poor discriminating power due to the small amount of ribosomal genes it contains.[111]. Flagellin typing is another rapid method for identifying *Campylobacter* spp. with excellent discriminatory power while using the restriction fragment length polymorphism (RFLP) technique [69, 104].

DNA sequencing is a practical alternative method for genotyping of bacterial isolates due to its automation improvement [104]. The MLST, a genotypic technique developed in 1991 [122], directly assigns DNA sequencing of 7 to 11 housekeeping genes and it provides different alleles in databases that can be compared [111]. This technique is the most frequently molecular typing approach used for campylobacters [108] and it is increasingly being utilized in epidemiological researches to characterize *C. jejuni* [123], *C. coli, C. lari* and *C. upsaliensis* [124]. However, the seven MLST loci may not be enough to give an accurate picture of the gene contents across the entire *C. jejuni* genome [125]. Moreover, there is a difficulty in distinguishing the closely related strains in short-term epidemic investigations. Therefore, additional technique such as *fla* typing may be essential in order to obtain sufficient resolution [105].

Comparative genomics, which involve analyzing and comparing two or more genomes have also served to underscore some of the new challenges in bacterial genotyping and phylogenetic analysis [108]. Another method for analyzing and comparing two or more genomes is comparative genomic fingerprinting (CGF) that is used to generate unique genomic fingerprints based on the differential carriage of accessory genes that can overcome some of the challenges in bacterial genotyping and phylogenetic analysis for genotyping purposes [108]. CFG-40 is a 40-gene comparative genomic fingerprinting approach for *C. jejuni* that has been confirmed to have stronger discriminatory power than MLST at both the clonal complex and sequence type levels for regular epidemiologic surveillance and epidemic investigations [126, 127].

Conclusion

Campylobacter is a foodborne pathogen associated with human gastroenteritis all over the world. Consequently, a better insight about its biology, virulence factor and sources of infection as well as early diagnosis using a variation of direct and indirect detection approaches are urgently needed to control the disease. Whereas applying the advanced typing and subtyping techniques in epidemiological investigations of campylobacteriosis, epidemics provide information to identify the possible sources of infection. Because no sole technique is efficient, evolving a novel typing method that incorporates efficiency and efficacy is vital to

avoid the defects of currently utilized methods.

Conflict of interest:

The authors declare no conflict of interest.

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الملخص العربي

أنواع الكامبيلوباكتر فى الدواجن: سمات الضراوة ، االية حدوث المرض ، التصنيف الوبائى وأهميته كمرض مشترك

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الكامبيلوباكتر هى بكتريا سالبة الجرام، منحنية على شكل حرف S، لا تستطيع تكوين حويصلة كما أنها متحركة بسوط قطبى واحد، و تعتبر الكامبيلوباكتر واحدة من أهم مسببات إلتهاب المعدة والأمعاء للإنسان والتى تنتقل عن طريق الغذاء. تستعمر الكامبيلوباكتر الجهاز الهضمى لمجموعة متنوعة من الحيوانات الأليفة والبرية وخاصة الدجاج و الرومى و الخنازير والذين يعتبرون العائل الرئيسى للكامبيلوباكتر. تنتقل العدوى إلى الإنسان، بشكل رئيسي من خلال تناول لحوم الدواجن الملوثة والحليب غير المبستر والمياه الملوثة ، مما يتسبب في آلام شديدة في البطن وحمى وإرهاق وإسهال. على الرغم من أهمية جنس الكامبيلوباكتر،هناك القليل من المعلومات المتوفرة حول خصائص تلك البكتريا ولذلك فإن هذه الدراسة المرجعية تتناول دراسة بيولوجيا البكتيريا وبقائها وخصائص نموها والعوامل المتعلقة بإمراضها وآليات حدوث المرضفى ضوء نتائج الدراسات السابقة . علاوة على ذلك ، قمنا بتوضيح العديد من التقنيات المستخدمة في التصنيف الوبائي لجنس الكامبيلوباكتر.