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REVIEW ARTICLE

Foodborne *Campylobacter* species: Taxonomy, Isolation, Virulence Attributes and Antimicrobial Resistance

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Abstract

Campylobacter is widely regarded as the main cause of foodborne diarrheal diseases worldwide. It is a curved Gram-negative rod displaying corkscrew motility via a polar non-sheathed flagellum. Campylobacter grows microaerophilically at a broad range of temperature (30–45°C), and it is considered biochemically inert. Campylobacter did not use carbohydrates to obtain energy because of lacking the 6-phosphofructokinase enzyme. Campylobacter has a positive reaction for oxidase test and a negative reaction for indole test. Laboratory isolation and detection of *Campylobacter* species is tricky as they are fastidious and necessitate special atmospheric requirements to grow. Relatively little information is known about the virulence attributes in campylobacters or how a seemingly fragile bacteria can survive with increased pathogenicity. Moreover, the growing antimicrobial resistance of campylobacters to the clinically crucial antibiotics becomes insurmountable. Thereby, this review elucidates and discusses the taxonomy, isolation, identification, virulence attributes and the antimicrobials resistance of this particular bacterium.

Keywords: *Campylobacter* spp., Isolation, Virulence factors, Antimicrobial susceptibility.

Introduction

Thermophilic *Campylobacter* species (spp.) is one of the highly significant four microorganisms causing diarrheal diseases universally. It is regarded as the most frequent cause of human gastroenteritis globally causing a disease called campylobacteriosis. Most infections occurred in humans are attributed to *C. jejuni* (80-85%), while the residual cases are credited with *C. coli* [1]. This bacterium is transmitted to people through contaminated undercooked foods, especially undercooked poultry meat and unpasteurized dairy products [2]. Despite, comparatively few reports about *Campylobacter* spp. virulence; these microorganisms have various virulence factors in regard to motility, adhesion, invasion and toxin-activity, among others [3]. Campylobacteriosis symptoms ranged from mild diarrhea to sever serious complications [4]. Human campylobacteriosis are mostly self-limiting. Despite that, patients with immunosuppression and young age with severe infections may need antimicrobial medications [5]. Erythromycin is generally the first drug of choice, where fluoroquinolones and on a smaller scale tetracycline constitute other options [6]. This review centered on the family's taxonomic history and their general characteristics. Moreover, it discusses the isolation, identification and confirmation of campylobacters, their virulence attributes in addition to their mechanisms of resistance to various antimicrobial classes.

Historical standpoint of *Campylobacter* species

Historically in 1886, the first written report regarding campylobacter is supposed to be introduced by Theodore Escherich, he noticed and prescribed a non-cultivable bacterium with a spiral shape [7]. After that, in 1906, campylobacter was firstly detected by 2 British veterinarians; Stewart Stockman and John McFadyean, who declared a huge number of peculiar organisms that were present in the

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uterus of a pregnant ewe [8]. The described organism was probably to be *Campylobacter fetus* [9]. Later, in 1913, MacFadyean and Stockman recorded a vibrio-like organism from fetuses of aborted ewes and termed it *Vibrio fetus*, which was recovered from bovine aborted fetus in 1919 [10]. In 1927, a bunch of bacteria like vibrio was discovered in feces of cattle suffering from diarrhea. They were detected by Marion Orcutt and Theobold Smith [11]. In 1931, a vibrio organism from calves and cows having winter dysentery was isolated by Jones et al., Later; the organism was termed as *Vibrio jejuni* [12]. In 1944, another vibrio was isolated by Doyle from pigs' feces suffering from diarrhea and termed it as *Vibrio coli* [13, 14]. In 1957, King stated that *Vibrio fetus* was implicated in humans with bloodstream infections [15]. Campylobacters were firstly classified as *Vibrio* spp. on grounds of their spiral shape. In 1963, Sebald and Veron have created the Campylobacter genus to distinguish them from members of Vibrio genus relying on the requirements for the microaerobic growth conditions, the low G and C base of their DNA, non-fermentative metabolism and elevated growth temperature [16]. During 1973, Chatelain and Veron incorporated 4 different species in Campylobacter genus: *C. sputorum*, *C. fetus*, *C. coli* and *C. jejuni* [17].

Taxonomy of *Campylobacter* species

Campylobacter spp. belongs to the epsilon subdivision of proteobacteria [18] and the order *Campylobacterales* [19]. The family *Campylobacteraceae* is a various group of environmental, commensal and pathogenic Gram-negative bacteria. At the present time, it has 3 genera: *Sulfurospirillum* (7 taxa), *Arcobacter* (17 taxa) and *Campylobacter* (30 taxa) [20]. From 2000 to 2009, many novel species were discovered and added to campylobacter taxa. *C. lanienae* [21], *C. hominis* [22], *C. hyolei* was changed to *C. coli* [23], *C. insulaenigrae* [24], *C. canadensis* [25], *C. avium* [26], *C. cuniculorum* [27] and *C. peloridis* [28]. Recently, from 2010 to 2015, six species were added; *B. ureolyticus* was changed to *C. ureolyticus* [7], *C. subantarcticus* [29], *C. volucris* [29], *C. troglodytes* [30], *C. corcagensis* [31] and *C. iquaniorum* [32]. Currently, the genus Campylobacter contains 31 species and 10 subspecies (http://www.bacterio.net/campylobacter.html, accessed 18.03.2018). *C. ornithocola* [33], *C. pinnipediorum* subsp. *caledonicus*, *C. pinnipediorum* subsp. *pinnipediorum* [34], *C. geochelonis* [35] and *C. hepaticus* [36] are the latest identified species.

General characteristics of *Campylobacter* species

In spite of variations in the host and source association, members of genus Campylobacter have multiple basic similar features. They are Gram-negative, curved bacteria. When two campylobacters converge together, they create the “S” shape as the wing of the gull. Cells are about 0.2–0.8 μm in width and 0.5–5 μm in length [37]. Under the microscope, cells of certain species are straighter rods (i.e., *C. showae*) and in *C. jejuni* strains, the cells are usually straight bacilli [38]. When cultures become old or undergo environmental stress conditions such as low nutrient, atmospheric oxygen and high temperature or freezing, cells can turn to coccal or spherical forms [20]. Coccoid forms are usually referred as viable, but non cultivable (VBNC) cells [4].

Campylobacter spp. are motile by a polar non-sheathed flagellum. The campylobacter helical shape together with their flagella generates a characteristic darting corkscrew-like motion under the phase-contrast microscopy [7]. There are two exclusions for campylobacter motility; *C. showae* has a tuft of flagella at one pole and *C. gracilis* and *C. hominis* have no flagella [37].

Campylobacter spp. proliferates at a temperature range of 30–45°C [39]. Thermophilic campylobacters (e.g. *C. upsaliensis*, *C. coli*, *C. jejuni*, and *C. lari*) have a better growth at 42°C and 37°C, but they can't grow at 25°C. However, other species have an optimum growth at 37°C [37]. Multiplication of *Campylobacter* spp. can be inhibited at temperatures less than 30°C. Therefore, the number of campylobacters in foods doesn't increase at room temperature (20–25°C). Despite the inability of

Campylobacters spp. to grow at temperatures less than 30°C, it can survive at 4°C within humid conditions [40]. Despite good surviving in low temperatures, *Campylobacter* spp. are heat sensitive and are inactivated by household cooking or pasteurization. *Campylobacter* spp. are destroyed immediately by heating at 55–60°C for few minutes [41].

Campylobacter spp. growth can be restricted by storage at -15°C or by NaCl of a concentration more than 2% w/v. Nevertheless, freezing cannot eliminate campylobacters. Furthermore, they are incapable of surviving at pH below 4.9 and above 9.0 [42]. Campylobacter sensitivity to ionizing radiation is greater than *Salmonella* and *Listeria* species [43].

Most *Campylobacter* spp. grows microaerophilically. However, some species can proliferate aerobically otherwise anaerobically. The dominant species, *C. coli* and *C. jejuni* need the microaerobic atmosphere (3–5% CO_2, 5–10% O_2 and 85% N_2) to grow [44]. *Campylobacter jejuni* can adapt the aerobic atmosphere as it can make biofilms. This improves its ability for surviving and spreading in food processing environments e.g. poultry processing [45].

Campylobacter spp. is somewhat inactive in the biochemical reactions. They did not use carbohydrates to obtain energy, but they have a respiratory metabolism using intermediates of the tricarboxylic acid cycle in addition to amino acids. This is because of lacking the 6-phosphofructokinase enzyme, which is contributed in the energy metabolism [46]. Traditional biochemical reactions of *Campylobacter* spp. involve the fumarate reduction to succinate, the positive reaction for oxidase test (except *C. gracilis*) and the negative reaction for indole test. Campylobacters can reduce nitrate with the exception of *C. jejuni* subsp. *doylei*. Lecithinase and lipase enzymes are absent and this genus can't hydrolyze starch, gelatin and casein [47]. Moreover, they are positive for catalase test except *C. upsaliensis* [48].

Global epidemiology of campylobacter infection

According to the Centers for Disease Control and Prevention (CDC) report, infection cases caused by campylobacters are 14 per year per 100,000 of the population in United Sates of America [49]. The problem of infections has increased 30 times more reported by the data on an outbreak [50]. Reports from South America also highlighted its increased prevalence, i.e. 4.6 to 30.1% in *C. jejuni* infections, while three studies from Argentina showed 0 to 1.4% prevalence rates of *C. coli*. The range between 4.4 to 10.5% of *C. jejuni* infections was reported from Bolivia. The campylobacter infection ranges were 0-14.1% in Chile , 0-14.4% in Colombia, 0-23.0% in Ecuador, 0.6-18.4% in Paraguay, 0-23.0% in Peru, 0-14.3% in Uruguay and 0-13.0% in Venezuela [51].

A report evaluation in the European Union showed high prevalence rates of campylobacter infections in Bulgaria; i.e. 13,500 cases per 100,000 population, while less incidence rates in Finland and Sweden were reported [52]. In the United Kingdom, the rate of campylobacter infection was 9.3% per 1000 cases per year [53]. In Germany, campylobacteriosis incidence was 53.4-81.4% per 100,000 of the population [54]. The reports from China showed 4.84% prevalence rate of *C. jejuni* infections with 14.9% of patients suffering from gastroenteritis in Beijing [19, 20]. In Japan, campylobacteriosis was found to be 100 cases per 100,000 of population each year [55]. In India, patients having gastroenteritis were found to be culture positive for campylobacter; *C. jejuni* was found to be isolated from 70% of the reported cases, while another study accounted 16.2% of cases for various *Campylobacter* species [56, 57].

Prevalence of *Campylobacter* species in Egypt

In 1995, *Campylobacter* spp. was isolated from 880 (16.8%) children with diarrhea and from 1079 (6.4%) healthy children. The isolation of *Campylobacter* species was more frequent than *Salmonella*, *Shigella* and other bacterial enteric pathogenic species [58]. From

1986-1993 in Abbassia Fever Hospital, Cairo, Egypt, examination of 6,278 patients with acute enteric infections revealed the isolation of 92 strains (63%) of *C. jejuni* and 54 (37%) of *C. coli* [59]. In 2005, military personnel with diarrhea were participated in a military exercise in the northwestern Egyptian desert and the pathogens causing diarrhea were identified in 53.6% of 129 enrolled cases [60]. A similar study was conducted in 2011 on 72 personnel with traveler's diarrhea in Multinational Force and Observers in the Southern Sinai, Egypt and the bacteriological examination of their stool revealed the isolation of *C. jejuni* from 7 (10%) of the examined samples [61]. Later on, *Campylobacter* spp. were identified from patients with gastroenteritis in Cairo, Egypt using conventional and polymerase chain reaction (PCR) methods in 6.6% of human stool samples [62].

Importance of *Campylobacter* species in veterinary fields

Campylobacter species are almost ubiquitously in the environment and they are found in the intestinal tract of many wild and domestic warm-blooded animals, usually without showing clinical symptoms [63].

Birds are naturally infected via the fecal-oral route and ingestion of campylobacters numbers as few as 35 CFU can be sufficient for successful colonization [64]. The organisms colonize primarily the ceca and the colon and to a lesser extent the small intestine [65]. Young birds (less than 2–3 weeks) are rarely infected with campylobacter due to the maternal antibodies [66]. The colonization of the intestine has been associated with jejunal histomorphological changes, higher intestinal permeability, altered intestinal electrolyte transport with a decrease in the intestinal nutrient absorption and intracellular Ca2+signaling interference [67].

In cattle and sheep, *Campylobacter* spp. cause enteritis and abortion. They include *C. jejuni*, *C. fetus* subsp *fetus*, *C. hyointestinalis* subsp *hyointestinalis*, and *C. sputorum*, which causes abortions in sheep. However, in studies that compared *C. jejuni* prevalence in healthy cattle and in cattle considered sick because of diarrhea, the frequency of *Campylobacter* spp. was not significantly different. Beef and dairy cattle can have significant levels of campylobacters, with prevalence rates of 2.5%–60%.

In weaning aged pigs, campylobacters can contribute to colitis. Swine commonly carry *C. coli* and *C. jejuni* as intestinal commensals. Studies in the USA, Netherlands, Great Britain, and Germany showed that more than half of the commercially raised pigs excreted the organisms. *C. coli* strains comprise the most prevalent isolates from pigs causing first watery and then inflammatory diarrheal diseases. Pigs have anorexia, fever, and diarrhea for 1–5 days, followed by remission of clinical signs, but they continue to shed *C. jejuni* in their feces. *C. hyointestinalis* subsp *hyointestinalis* and *C. mucosalis* are also implicated as causes of enteritis in pigs. In pet animals, *C. jejuni* causes diarrhea in dogs and cats, which is considered a significant source of the bacterium for the human population. Diarrhea lasting 5–15 days is the most common clinical sign in dogs <6 months old. Occasionally, the diarrhea becomes chronic and may be accompanied by fever and increased the white blood cells` counts [68].

Zoonotic importance of *Campylobacter* species

Human infection with campylobacter, leading to campylobacteriosis, is caused by the thermophilic members of the genus *Campylobacter*; *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*. Interestingly, *C. jejuni and C. coli* are the most frequently identified species associated with food borne illness. They together account for approximately 95% of human campylobacteriosis cases worldwide [69]. An infective dose as low as 500-800 live campylobacter cells may be sufficient to cause an illness in humans [70].

Generally, the human campylobacter infections are considered foodborne. It occurs via consumption of undercooked meat and meat products, mainly poultry as well as raw or contaminated milk and contaminated untreated water. Contact with pet animals has also been proposed as a mode of transmission of campylobacteriosis in humans [71].

Campylobacter is also recovered from seawater and bovine manure compost [37]. Campylobacteriosis is an acute self-limiting foodborne enteric illness. The incubation period ranges from 2-5 days, but it has been reported to be up to 10 days. In most patients, symptoms include diarrhea, abdominal cramps, myalgia, fever and occasionally vomiting [72]. Additionally, serious post-infectious complications such as Guillain-Barré syndrome and reactive arthritis may be occurred in 0.1% and 1% of the population, respectively [73].

Isolation and identification of *Campylobacter* species

Isolation of Campylobacter species

Laboratory isolation and identification of *Campylobacter* species is laborious as they are fastidious and necessitate special atmospheric requirements to grow. The isolation step is further compounded by the existence of commensal bacteria that grow rapidly and vying with campylobacter on the medium [74].

All *Campylobacter* spp. selective media have scavengers of oxygen such as charcoal, blood, and ferrous iron as campylobacter is sensible to oxygen. The selective agents are particularly a mixture of antibiotics to which campylobacter are resistant e.g vancomycin, colistin, cycloheximide and nystatin. These antibiotics suppress the proliferation of several microbial flora exist in samples and therefore allowing the *Campylobacter* spp. to proliferate [75, 76].

Methods of campylobacter isolation mostly necessitate a pre-enrichment in broth before plating on the agar plates [77]. Campylobacter isolation from faecal samples is done by plating directly on selective media followed by microaerobic incubation at 42°C [78] as feces frequently contain viable campylobacters with high number [79]. However, environmental samples and food products usually have few stressed cells; thus, the initially enrichment step in the liquid medium is required [77].

The first utilized selective medium for campylobacter culturing was reported by Skirrow. After that, more than forty liquid and solid selective culture media have been listed and evaluated [80].

Multiple enrichment broths such as Bolton broth, Preston broth and Campylobacter enrichment broth have been tested for their effectiveness [81]. Solid media such as Preston, charchoal cefoperazone deoxycholate (CCDA) and Butzler agars have been proved to possess the same effectiveness [82]. The latest standard method [9] for isolation and detection of *Campylobacter* spp. uses CCDA as the selective medium, while Bolton broth is utilized for the enrichment step. Moreover, the microaerobic incubation at 42°C is typically the preferred method as it enables more campylobacter strains to proliferate [83]. Alternative combinations of enrichment broth and plating were used to detect and enumerate campylobacters in meat of chicken [84] and they look like to give significantly better results.

Identification of Campylobacter species

Among the *Campylobacter* spp. growing at 42°C, *C. coli* and *C. jejuni* are the most common emerged species from samples; however, low frequencies of other species were also reported. Speciation is difficult due to the consequence of the complicated and the swiftly changing taxonomy in addition to the inertness of *Campylobacter* spp. to biochemical tests. These problems have generated phenotypic and genotypic methods to distinguish the members of that group [60].

Campylobacters are biochemically inactive compared with other bacteria. As a consequence, the phenotypic tests used to differentiate them to the species level are few. In general, *C. jejuni* can be distinguished from others relying on hippurate hydrolysis as it is the only species that have the hippuricase gene giving a hippurate positive result. However, variability in the hippurate reaction has been noticed in some of *C. jejuni* strains resulting in hippurate-negative results [86]. There are other biochemical tests used in species identification such as catalase test, which is negative in *C. upsaliensis* and indoxyl acetate hydrolysis test, which is negative in *C. lari* [9].

In earlier times, nalidixic acid and cephalothin susceptibility testing have been relied on for the species identification [87]. Both *C. coli* and *C. jejuni* are cephalothin

resistant [78]. In contrast, *C. upsaliensis* is sensitive to cephalothin [9]. Nowadays, the sensitivity to nalidixic acid may yield difficulties in the interpretation [88] due to the increasing resistance to fluoroquinolones [89]. Therefore, antimicrobial susceptibility tests cannot be utilized for the phenotypic identification of campylobacter isolates [85]. As a consequence of the difficulties and the inaccuracy of the phenotypic identification, multiple molecular methods were used to supplement the biochemical tests or to replace them [90]. Detecting the species -specific sequences using PCR is auxiliary; specifically, when the distinction between hippuricase negative *C. jejuni* strains and *C. coli* is required and using biochemical tests alone is inadequate [91].

Campylobacter species virulence factors

Specific virulence mechanisms are not clearly demonstrated for *Campylobacter* species up till now, probably owing to the paucity of pathogenesis resemblance between campylobacters and the other bacteria [92]. Bacterial motility, adherence, invasion ability and toxins production have been described as virulence factors for *Campylobacter* spp. [92, 93] (Figure 1) [94] as following:

Motility

Motility is important to get away from the stressful environments [95]. Campylobacter motility system requires a chemosensory system and flagella that drives the bacterial movement in reliance of the environmental conditions. The spiral shape of campylobacter in addition to its flagella allow it to swim through the thick mucus layer covering the intestine enabling the campylobacter to reach efficiently to their favored site of colonization. This system is formed by many proteins, which have different roles e.g. the main flagellum filament proteins, FlaA (the major flagellin subunits) and FlaB (the minor subunit), those were encoded by *flaA* and *flaB* genes, respectively [3]. Other crucial protein, CheY that is a response regulator demanded the flagellar rotation [96].

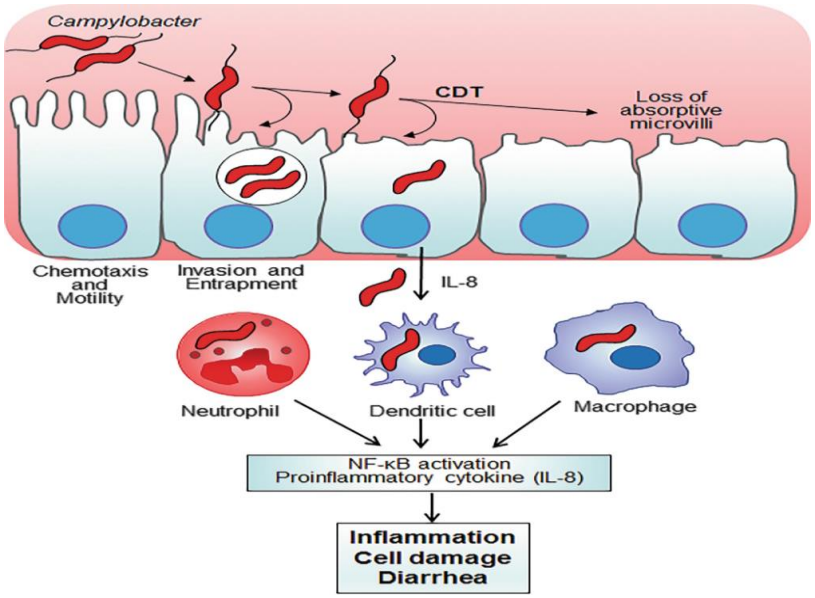

Figure 1: Diagram demonstrates the virulence attributes of *Campylobacter* species taken from a previos report [94]. IL-8: interleukin-8, NF-Kb: nuclear factor kappa-light-chain-enhancer of activated B cells

The bacterial flagellum is also relevant with bacterial adhesion and invasion into the host cells. Campylobacter flagellum has components analogous to the classical Type III secretion systems (T3SS) that assist in the transport of the non-flagellar proteins (FlaC, FspA, CiaB, CiaC and CiaI) [97] playing a pivotal role in campylobacter pathogenesis [98].

Additional to their essential role in bacterial mobility, it can modulate the host responses through Toll-like receptor 5 (TLR5). The TLR5 usually triggers a potent host response, which gives critically important signals to maintain the immune homeostasis of intestine. Despite that, *C. jejuni* can escapes from the recognition of TLR5 because of the structure of its flagellin protein is clearly different from other bacterial flagellins stimulating this system [99].

Adhesion

The capability of campylobacter to adhere to the epithelial cells of the host gastrointestinal tract is a fundamental precondition for colonization. This process is mediated by variety of adhesins existing on the bacterial surface; in addition, it is essential to generate a disease [100].

Adhesion of campylobacter to fibronectin is brokered by CadF (fibronectin binding outer membrane protein) [101], that is encoded by the chromosomal *cadF* gene [102]. Binding to fibronectin (glycoprotein found in GIT epithelial cells) stimulates signals that lead to an activation of Cdc42 and GTPases Rac1 that spur the campylobacter cell internalization. Many studies with mutants exhibited that the absence of this protein inhibits the campylobacter colonization [103]. Furthermore, some proteins were recognized in the colonization process e.g. CapA (auto transporter), PEB1 (periplasmic binding protein) and JlpA (surface-exposed lipoprotein) [3]. Initially, the FlpA was regarded to be important for binding to epithelial cells of poultry intestine [104]. However, other studies proposed that FlpA is capable of binding to fibronectin [105] and it is proposed that CadF plus FlpA work together during the adhesion and the subsequent invasion of *Campylobacter* spp. [106].

Indeed, inhibitor reports with chloramphenicol, that retards the Cia protein expression, but not FlpA or CadF, propose that invasion of the host cell necessitates more of a constitutive expression of the last 2 adhesins [106]. It is noticed that there was a correlation between the degree of adherence of campylobacters to host cells and the extreme of the clinical manifestations in infected peoples [92].

Invasion

Campylobacter invasion ability is a vital pathogenicity factor. The clinical manifestations of campylobacteriosis are consistent with the cellular invasion. A variety of proteins termed campylobacter invasion antigens (Cia), are considered the key for invasion to host cell and survival, they are transferred to the host cells cytosol by a flagellar T3SS [107]. In that regard, flagellar mutants have considerably reduced the invasiveness ability of campylobacters [101]. There are 3 Cia proteins; CiaB, which is needed for target cells adherence, CiaC, which is concerned with complete invasion of cells and CiaI, which is requisite for surviving within cells. Recently, CiaD has been detected as an essential factor needed for the best invasion of cell hosts [108].

Moreover, the CiaB mutants have decreased the campylobacter invasiveness ability by a marked decrease in their adherence capability. Other proteins e.g. FspA, IamA, FlaC, VirK, CeuE and HtrA have been proposed to play a role in host cells invasion, however the right mechanisms are not understood probably up till now [3].

Toxin production

Cytolethal distending toxin (CDT) is broadly spread among Gram negative bacteria [109]. It is regarded to be the best toxin generated by *Campylobacter* spp. It is a serious virulence factor that has been listed for this pathogen [110]. *Campylobacter* spp. including *C. lari, C. jejuni, C. coli, C. fetus* subsp. *fetus, C. fetus* subsp. *venerealis, C. hyointestinalis* and *C. upsaliensis* can produce toxins [111].

The CDT is comprised of 3 subunits: CdtA, CdtB and CdtC. The CdtA coupled with CdtC are accountable for the binding and the internalization into host cells, while the CdtB subunit is the toxic subunit, which is driven into the cell nucleus and induces cleavage in the DNA, arrest in cell cycle followed by cell death [3, 92, 112]. In fact, all the three products of the *cdt* gene should be existed together to be functionally active [110, 113].

The CDT activity differs somewhat relying on the affected eukaryotic cell exhibiting 3 types of avenues: (1) in keratinocytes, epithelial and endothelial cells subjected to G2 cell cycle arrest and death, (2) the fibroblasts subjected to G1 and G2 arrest, cell distension and death and (3) the immune cells subjected to G2 arrest with subsequent apoptosis. Because of these actions, CDT participates in campylobacter pathogenesis through inhibiting both humoral and cellular immunity through apoptosis of the cell's immune response [114]. Any changes occur at the level of actin and microtubules filaments could block the toxin; therefore, it cannot reach the nucleus and thereby it prevents the DNA damage and leads to alterations in cell cycle [115].

Antimicrobial resistance mechanisms of *Campylobacter* species

Campylobacter is a commensal bacterium of many farm animal species so, it exposed to high number of veterinary antibiotics. Moreover, the hypervariable genomic sequences and the natural competence of campylobacter are conferring for its substantial genomic plasticity, which responsible for these resistances [116].

Several mechanisms have been implicated in the development of antimicrobial resistance such as (i) production of inactivating enzymes to antibiotics, (ii) protection or alternations of the antibiotic targets, (iii) extrusion of drugs away from the bacterial cells via the active efflux transporters and (iv) decreasing the permeability to antibiotics. Some of campylobacter resistance traits are endogenous, while others acquired either by the genetic transfer or mutations [117].

Campylobacter spp. has an intrinsic resistance to sulfamethoxazole, cloxacillin, trimethoprim, oxacillin, nafcillin and vancomycin. However, other resistance types occur during the use of therapeutic antimicrobials in humans and animals [118]. Antimicrobial resistance mechanisms in campylobacters are well documented for many antimicrobial classes as following:

Aminoglycosides

Aminoglycosides are bacterial protein synthesis inhibitors which execute their antimicrobial activities through binding to the 30S ribosome. The principle mechanism of campylobacter resistance to aminoglycoside is through the plasmid borne aminoglycoside-modifying enzymes (AphD, AphA, Sat and AadE). Meanwhile, the efflux contribution to their resistance is unclear up till now [96, 116].

Tetracyclines

]Tetracyclines are antimicrobial agents that are utilized frequently in veterinary and human medicine [119]. These agents inhibit the elongation process of protein synthesis through binding to the bacterial 16S rRNA within the ribosome minor subunit [120]. Campylobacter resist tetracyclines by expressing one of these mechanisms: alteration in tetracycline ribosomal target, 16Sr RNA and efflux pumps [121]. The most common distributing mechanism among *Campylobacter* spp. is through protecting the binding site between the ribosome and the antimicrobial agents. This is brokered by the TetO protein, which is encoded by *tet (O)* gene. The TetO protein binds to the ribosomal A site leading to separation of the attached tetracycline [117]. The *tet (O)* gene in most strains is encoded by plasmid. In contrast, few numbers of isolates have a chromosomal copy of this gene [122].

β-Lactams

The β-lactam ring in β-lactam antibiotics is needed for the antimicrobial activity. β-lactam antibiotics inhibit the cell wall biosynthesis by binding to the bacterial peptidoglycan transpeptidases resulting in lacking the structural integrity of their walls [116]. There are 2 main mechanisms responsible for campylobacter β-lactam resistance: (1) the enzymatic inactivation by β-lactamases enzymes and (2) efflux pumps [116]. In campylobacters the expression of β-lactamases

accounts for the resistance to ticarcillin, ampicillin and amoxicillin, that is may be antagonized by clavulanic acid, sulbactam and tazobactam. These enzymes have no effect on the susceptibility to carbapenems and cephalosporins [116]. Lately, OXA61 β - lactamase has been discovered in *C. jejuni* [123]. This enzyme is responsible for the resistance to piperacillin, oxacillin, ampicillin, penicillin, carbenicillin and amoxicillin-clavulanate [116]. Moreover, the CmeABC efflux pump has been participating in campylobacter resistance to ampicillin. Insertional mutagenesis of *cmeB* gene leads to increasing in the ampicillin susceptibility. Alterations in the porins of the outer membrane have a potential mechanism of resistance to β-lactam antibiotics, even so the underlying mechanism is still not described clearly [124]. These antibiotics are applied widely in veterinary medicine; however, their emerging resistance has compromised their use.

Quinolones

In campylobacters, quinolones inhibit the DNA gyrase enzyme that is needed for DNA supercoiling and replication [124]. *Campylobacter* species resist quinolones via a single mutation in the *gyrA* gene and by the CmeABC efflux pump activity [116]. Many different single modifications in *gyrA* were reported in *Campylobacter* species such as Thr86Ile, Thr86Lys and Thr86Ala. However, the more frequent one is the Thr86Ile substitution. This mutation accounts for the high campylobacter resistance level to this group [124]. In all European State members, human campylobacter isolates showed high ciprofloxacin resistance levels. Consequently, fluoroquinolones have ceased to be the proper drug for routine treating of campylobacterosis [125]. Many reports have demonstrated a clear correlation between using fluoroquinolone in avian and the increasing resistance in avian and human campylobacter isolates [117].

Macrolides

Historically, the macrolides resistance incidence was low, especially in *C. jejuni*. However, in last years, macrolides resistance in campylobacters has increased. Moreover, it is traditionally reported in multiple European Union members at higher levels [125]. Campylobacters acquire resistance to these agents by 4 mechanisms; (i) mutations in 23S rRNA genes, (ii) ribosomal proteins mutations, (iii) ribosomal methylation encoded by *erm (B)* gene and (iv) efflux through CmeABC [126]. Lately, the previously mentioned third way has been detected in Spain in a *C. coli* isolate from broilers. This was the first record in Europe recording the existence of *erm (B)* in *Campylobacter* species [125]. Macrolides work via targeting the 50S subunit of bacterial ribosome in addition to inhibiting the bacterial RNA-dependent protein synthesis. Moreover, the 23S rRNA nucleotides 2059 and 2058 serve as key binding sites for macrolide. This results in ribosomal conformational changes followed by ending the peptide chain elongation process. In campylobacter chromosome, 3 copies of 23S rRNA gene were found and generally in strains resistant to erythromycin. All copies possess macrolide resistance-associated mutations; however, presence of wild-type alleles doesn't seem like to have an impact on the level of resistance [124]. Moreover, efflux pumps are considered another resistance mechanism. In campylobacter, at least 8 efflux systems were described, but CmeABC pump is the virtually relevant efflux pump, which operates in a synergistic manner with mutations in 23S rRNA gene attributing for the high resistance toward macrolides [127].

Multidrug efflux pump system

The efflux pumps in *Campylobacter* spp. cooperate with the other mechanisms of resistance. They are related to the intrinsic resistance mechanisms toward a broad range of antibiotics. Variety types of these pumps were found in campylobacters e.g. CmeABC (the best stated efflux pump), CmeDEF and CmeG [128]. CmeABC is a resistance-nodulation cell division (RND) type of multidrug efflux pump. It is composed of 3 proteins; CmeA (periplasmic fusion protein), CmeB (inner membrane drug transporter) and CmeC (outer membrane protein) [100]. CmeABC expression is modulated by CmeR, which inhibit *cj0561c* gene. The CmeABC has

a fundamental contribution in the antimicrobial resistance of campylobacters attributed to that the *cmeB* inactivation or using an inhibitor to efflux pump induce increasing in susceptibility to various antibiotics even the antimicrobial agent to which the campylobacters are intrinsically resistant [129].

Modern and new molecular techniques to detect the virulence and antibiotic resistance genes

Variations of PCR have been developed and employed to detect virulence and resistance genes such as multiplex PCR [130] and enter bacterial repetitive intergenic consensus PCR (ERIC-PCR) analyses [131]. Identification of virulence factors successively relied on biochemical approaches, or systematic molecular screening of a panel of genes demonstrated to play a role in pathogenesis using molecular cloning and/or mutagenesis [132]. In addition, several multi-criteria genome analysis tools enable detecting virulence factors. The MvirDB combines several databases including PRINTS and VFDB to identify virulence factors [133]; the Pathosystems resource integration center (PATRIC) combines the VFDB, Victors and PATRIC VF databases to detect virulence factors and host–pathogen protein–protein interactions [134] and the PHI-base (pathogen–host interaction) database can identify virulence factors and host–pathogen protein–protein interactions also enables detecting 2875 virulence genes and 4102 host–pathogen interactions [135].

Multilocus sequence typing (MLST), based on sequence comparison of seven housekeeping genes defined as sequence types (STs) and clonal complexes (CCs), has been an essential tool in studying of C. jejuni phylogeny and epidemiology [136]. However, MLST does not include medically relevant information such as the virulence or antibiotic resistance determinants, also known as virulome and resistome [136]. In addition, since *C. jejuni* is genetically variable pathogen with high level of horizontal gene exchange and recombination, even strains representing the same STs may possess distinct virulence patterns [137]. At present, whole-genome sequencing (WGS) is considered as the most informative and discriminative typing method of bacterial pathogens, allowing for comprehensive phylogenetic analyses of numerous traits associated with virulence or antibiotic resistance [138].

Conclusion

Despite campylobacteriosis is a disease of zoonotic importance in Egypt, there is a gap of knowledge about the disease's epidemiology in different localities, which hinders the accurate assessment of the human health burden. There is an urgent need for collaborative surveillance and intervention national plans for controlling such infection. From the standpoint of one health approach, a complete health surveillance program of campylobacter infections must be done nationally to provide data about the disease occurrence and the common routes of transmission. Notifications of the disease in all suffering regions should be happen rapidly. As well, it is very important to collect, analyze and interpret data to create relationships between campylobacter isolates of human and those of animal origins. Raising the public health awareness, education and training of the target populations (veterinarians, farm, abattoir and restaurant workers, household, nurses, doctors in hospitals, etc…) are very crucial. Biosecurity measures, vaccination or using natural competitive exclusion compounds are very critical to reduce the risk of infection in the farms and consequently reduce the level of transmission to humans.

Conflict of interest

None of the authors have any conflict of interest to declare

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الملخص العربي

تسميه, عزل, سمات الضراوة و المقاومة للمضادات الحيوية لانواع الكامبيلوباكتر المنقولة عن طريق الأغذية

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علي نطاق واسع, تعتبر الكامبيلوباكتر السبب الرئيسي لامراض الاسهال المنقولة عن طريق الاغذية في جميع انحاء العالم. فهي بكتريا سالبه الجرام تتحرك بواسطه سوط قطبي, تنمو فى وجود نسبة قليلة من الاوكسجين وفي نطاق واسع من درجه الحرارة من 30 الي 45 درجة مئوية. فضلاً عن ذلك فإن هذه البكتريا خاملة في الاختبارات الكيمائية ولا تستخدم الكربوهيدرات للحصول على الطاقة, حيث يرجع ذلك الي عدم وجود إنزيم 6 فوسفوفركتوكيناز. هذه البكتريا لديها تفاعل ايجابي في اختبار الاكسدة وتفاعل سلبي لاختبار الاندول. إن عزل وتشخيص أنواع الكامبيلوباكتر في المختبرات أمر صعب لأنها تنمو بصعوبة وتتطلب ظروف هوائية خاصة لنموها. وهناك معلومات قليلة نسبيا عن عوامل الضراوة الخاصة بالكامبيلوباكتر او كيف يمكن لبكتريا هشة ان تظل علي قيد الحياه مع زيادة الحاله المرضية. بالاضافة الي ان مقاومة الكامبيلوباكتر المتنامية للمضادات الحيوية السريرية اصبحت مستعصية. لذلك هذا المقال يوضح ويناقش تسمية وعزل وتصنيف وسمات الضراوه ومقاومة المضادات الحيوية لهذه البكتريا.