



Campylobacter in Poultry: Species Emergence, Pathogenesis and Antibiotic-Resistance Prevalence



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Abstract

Members of the genus *Campylobacter* are Gram-negative bacteria, microaerophilic which colonize the mucosal surfaces of the intestinal tracts of humans and animals. Many *Campylobacter* species belonging to this group are known pathogens in humans and animals and they are one of the most commonly reported causes of bacterial gastroenteritis in the worldwide. The aim of this review is to describe the principals physiological, biochemical traits of genus and the methods used for isolation, identification and detection of *Campylobacter* species and related organisms from food, and primarily poultry products. The relationship between the emergence and prevalence of antibiotic-resistance of *Campylobacter* spp., strains found in poultry problems in developed and developing countries and possible transmission to humans are also analyzed.

Keywords: *Campylobacter*; Emergence; Prevalence; Antimicrobial-Resistant; Poultry; Gastroenteritis; Identification

Introduction

The *Campylobacter* species are Gram-negative spiral, rod-shaped, or curved bacteria with a single polar flagellum, bipolar flagella, or no flagellum, depending on the species and microaerophilic [1]. The genus *Campylobacter* initially classified as *Vibrio* spp. due to their spiral morphology was first proposed by [2]. Member of this genus colonize the mucosal surfaces of the intestinal tracts, oral cavities, or urogenital tracts of a wide range of bird and animal hosts. *Campylobacter* spp. Are normal intestinal inhabitants of a wide variety of animals and avian species but frequently pathogens of humans. The members of *Campylobacter* are considered responsible for human bacterial enteritis and poultry meat is recognized as a primary source of infection [3]. The infection is most often acquired through consumption of contaminated food, in particular poultry products, however, other sources also contribute to human infections [4]. The incidence and prevalence of *Campylobacteriosis* (*Campylobacteriosis* is an enteric infection caused by members of the genus *Campylobacter* [5,6]) have increased in both developed and developing countries over the past 10 years and considered as the most commonly reported zoonotic bacterial disease within the European Union.

Many species of domestic poultry such as chickens, turkeys, ducks, geese, and wild birds are frequently infected with thermophilic *Campylobacter*, primarily *C. jejuni* [7]. Poultry products are considered one of the most important sources of protein for humans, especially in developing countries. In poultry, mainly in

broiler chickens, *C. jejuni* is the predominant species colonizing the flocks, followed by *C. coli* and rarely other species. Also, the ability to survive in low temperatures explains why refrigerated carcasses of poultry contaminated in the slaughter process are a common source of *C. jejuni* infections [8]. Today, numerous antibiotics are used for the treatment of infectious diseases. Some antibiotics agents are effective against only a limited range of infections bacteria (narrow spectrum); others are effective against a wider range (broad spectrum) [8]. The treatment with antibiotics has been shown to be beneficial in patients with gastroenteritis or other extra-gastrointestinal infections caused by emerging *Campylobacter* spp. However, the emergence of antibiotic-resistant *Campylobacter* constitutes a serious threat to public health. With the emergence of antibiotic-resistant *Campylobacter* constitutes a serious problem in public health with significant social, medical and economic consequences.

The aim of this review was describes:

- A. Taxonomy of *Campylobacter* genus including physiological, biochemical and molecular properties;
- B. The principals medium and methods used for identification of *Campylobacter* species from food, poultry products and stool samples;
- C. The most common human infections caused by the pathogenic species of *Campylobacter*

D. The relationship between the emergence and prevalence of antibiotic-resistance of *Campylobacter* spp. Strains found in poultry problems in developed and developing countries and possible transmission to humans.

Taxonomy and characteristics of *Campylobacter* spp

The early history of the genus *Campylobacter*, starting with the first description in 1886 by Theodor Escherich [9], who published series of articles in *Münchener Medizinische Wochenschrift*, in which he described spiral-shape bacteria in colon of children of what he called "Cholera infantum". The first isolation of a *Vibrio*-like organism from aborted ovine fetuses was by [9], who implicated these organisms as causal agents of abortion in sheep. A few years later [10] in the U.S. reported on the association of similar organisms with bovine abortion. *Campylobacters* were originally referred to as "micro-aerophilic vibrios" due to their spiral morphology [11,12] reported similar *Vibrio* from the blood cultures of humans with gastroenteritis [2] proposed the genus, *Campylobacter* ("curved rod" in Greek). They transferred these *Vibrio* species, *V. fetus* and *V. bubulus* into the new genus, *Campylobacter*, as *C. fetus* sp. nov., comb. nov., and *C. bubulus* sp. nov., comb. nov., respectively. However, the microaerophilic vibrios differed significantly from *V. cholerae* and certain other vibrios and vibrio-like organisms with respect to their biochemical and physiological properties, and DNA base composition analysis.

The genus *Campylobacter* was first proposed by [2], and included just two species, *C. fetus* and *C. bubulus*. *Campylobacter* genus belongs to the Proteobacteria phylum that consists of over 200 genera and represents the largest and most diverse group of organisms and contains the majority of Gram-negative species. This phylum is divided in subdivisions: Alpha-, Beta-, Gamma-, Delta-, and *Epsilonproteo* bacteria [13]. The *Campylobacter* genus belongs to the family *Campylobacteraceae*, the order *Campylobacterales*, the class *Epsilon proteobacteria*, and the phylum Proteobacteria. Since its first description, the genus has grown to include several important human and animal pathogens that are primarily classified through phylogenetic means. The family *Campylobacteraceae* comprises the genera *Campylobacter*, *Arcobacter*, and *Sulfur spirillum*, with average G+C content of the DNA between 29-47 [14]. The *Campylobacter* genus consists of a large and diverse group of bacteria, currently comprising more than 30 species and subspecies. The members of genus carry a relatively small genome that is a singular, circular chromosome of 1.59-1.77 MBP in size. All strains showed growth at 25 °C and 37 °C, but no growth at 42 °C. The only other members of the genus *Campylobacter* showing growth at 25 °C after 72h are *C. fetus* and *C. hyointestinalis* subsp. *Hyointestinalis* [15].

Most *C. fetus* subsp. *fetus*, but not *C. fetus* subsp. *venerealis* and all *C. hyointestinalis* strains are able to grow at 42 °C [16], the optimum growth temperature is 30 to 37 °C [17], in microaerobic to aerobic conditions. Strains show no hydrolysis of casein or gelatin; most strains do not hydrolyze urea. Isolated primarily from the reproductive organs and intestinal tract of man and animals [18], *Campylobacter* spp. are small, non spore forming, Gram-

negative bacteria that have a characteristic curved, S-shaped, or spirally curved rods, 0.2 to 0.8µm wide and 0.5 to 5µm long [19], nonsaccharolytic bacteria with microaerobic growth requirements and a low G+C content.. The genus *Campylobacter* was consisted in 26 species, 2 provisional species, and 9 subspecies, at present, the genus is recovering 28 species [16,20], with *C. fetus* as the type species [2]. Cells of most species are motile, with a characteristic cork-screw-like motion performed by means of a single polar unsheathed flagellum at one or both ends of the cell. Cells of some species are nonmotile (*C. gracilis*) or have multiple flagella (*C. showae*).

Most *Campylobacter* species have a respiratory type of metabolism and several species (*C. concisus*, *C. curvus*, *C. rectus*, *C. mucosalis*, *C.2m showed*, *C. gracilis*, and, to a certain extent, *C. hyointestinalis*) require hydrogen or format as an electron donor for microaerobic growth. In addition, several species grow in anaerobic conditions with fumarate or nitrate as electron acceptor; these species grow only in microaerobic conditions if hydrogen, format, or succinate is supplemented as electron source. Oxidase activity is present in all species except *C. gracilis*. Gelatin, casein, starch, and tyrosine are not hydrolyzed. Typical biochemical characteristics are reduction of fumarate to succinate, negative methyl red reaction and acetoin and indole production; and for most species, reduction of nitrate, and absence of hippurate hydrolysis. Actually, selective media are developed and can be used either for direct plating or for an enrichment step followed by plating for isolation of *Campylobacter*. Numerous types of enrichment broths have been developed for isolation of *Campylobacter* from foods [21].

Usually, enrichment broths consist of a basal medium, such as brucella-FBP (a combination of ferrous sulfate, sodium metabisulfite, and sodium pyruvate), or nutrient broth [21,22], modified charcoal cefoperazone deoxycholate, Park and Sanders, Bolton, Hunt and Radle, and Hunt broths, or supplemented with antimicrobials [21-23]. Also, other broth medium has been developed such as *Campylobacter* enrichment broth (CEB). The incorporation in broth medium, the enzyme Oxyrase is particularly effective in reducing the levels of oxygen or using different antibiotics in medium improving the isolation of *Campylobacter* spp. from naturally samples [24,25]. Many agar medium particularly Preston, Doyle and Roman, charcoal cefoperazone deoxycholate (CCDA), Butzler agars and CAT agar (Modified CCDA) [26], has been tested for isolating the *Campylobacter* from contaminated samples visible colonies usually appear on the plating media within 24-48h.

Methods of identification and typing of *Campylobacter* spp.

Members of *Campylobacter* can be distinguished from other microorganisms on the basis of several standard criteria and can be distinguished from one another on the basis of biochemical testing. But these tests have not found a practical application in laboratories because of their lack of reliability and time-consuming (Table 1 & 2). Today, several methods are used for identification of the strains belonging to the genus of *Campylobacter*. Among these techniques, the miniaturized most probable number (MMPN) methods, which

can be performed quickly, have been developed to facilitate enumeration of several target organisms [27-29]. This method has been developed by [30], for the enumeration of thermophilic *Campylobacter* spp. from critical poultry-associated reservoirs using a modification of blood-free Bolton broth (supplemented with 25mg/l of sulfamethoxazole) and Campy Food ID agar. This method

was based on a procedure developed by [29] for the enumeration of *Salmonella* from poultry matrices. The immunological methods are also currently used for identifying and detection of *Campylobacter* spp. in stool and food samples. Although, a variety of immunoassays have been developed for testing clinical and food samples for *Campylobacter* spp.

Table 1: Characteristics differentiating species of the genus *Campylobacter* according to Gilbert et al., (2015)

Characteristic	<i>Campylobacter</i> <i>terquianorum</i>	<i>C. avium</i>	<i>C. canadensis</i>	<i>C. coli</i>	<i>C. concisus</i>	<i>C. cuniculorum</i>	<i>C. curvus</i>	<i>C. fetus</i> <i>subsp. fetus</i>	<i>C. fetus</i> <i>subsp. venerealis</i>	<i>C. gracilis</i>	<i>C. helveticus</i>	<i>C. hominis</i>	<i>C. hyointestinalis</i> <i>subsp. hyointestinalis</i>	<i>C. hyointestinalis</i> <i>subsp. lowsonii</i>
Oxidase	+	+	+	+	v	+	+	+	+	-	+	+	+	+
Catalase	+	+	v	+	-	-	-	+	(+)	(-)	-	-	+	+
Urease	-	-	v	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	v	+	(-)	+	+	+	(+)	(+)	+	v	+	+
Hippurate hydrolysis	-	+	-	-	-	-	(-)	-	-	-	-	-	-	-
Indoxyl acetate hydrolysis	-	+	-	+	-	+	v	-	-	(+)	+	-	-	-
γ-Glutamyltransferase	+	-	+	-	-	-	ND	-	-	ND	-	ND	-	-
H ₂ S production (TSI)	+	-	v	-	-	-	(-)	-	-	-	-	-	+	+
α-Haemolysis	+	-	-	(-)	(-)	+	(-)	-	v	-	+	ND	v	v
Growth at/in/on: 18-22 °C (microaerobic)	+	ND	ND	-	-	ND	-	(+)	(-)	-	-	ND	(-)	-
25 °C (microaerobic)	+	-	-	-	-	-	-	+	+	-	-	-	(-)	-
37 °C (microaerobic)	+	+	+	+	+	+	v	+	+	-	+	+	+	+
42 °C (microaerobic)	-	+	+	+	(+)	(+)	v	(+)	-	v	+	(-)	+	+
37 °C (anaerobic)	+	-	+	-	+	-	+	(-)	v	+	-	+	-	+
37 °C (aerobic)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCDA	+	-	+	+	(-)	(+)	(+)	+	+	v	+	ND	+	+
Glycine (1%)	+	-	v	(+)	(-)	-	+	+	(-)	+	v	+	+	v
Resistance to: Nalidixic acid (30µg)	+	-	v	-	(+)	v	+	+	v	v	-	v	+	+
Cefalotin (30µg)	-	+	-	+	-	(+)	-	-	-	-	-	-	(-)	-
H ₂ requirement	-	v	-	-	+	-	+	-	-	+	-	+	v	v
S-layer present	-	-	-	-	-	-	-	+	+	-	-	-	-	-
DNA G+C content (mol%)	36	35	nd	31	37-41	32	45-46	33-35	33-34	44-46	34	32-33	35-36	31-33
Oxidase	+	+	+	+	+	+	+	+	+	v	+	+	+	+
Catalase	+	v	+	+	+	+	-	+	(-)	+	v**	+	-	+
Urease	-	-	-	-	-	-	-	ND	-	-	v***	ND	-	ND
Nitrate reduction	-	-	+	+	+	+	(-)	ND	+	+	(+)	+	+	+
Hippurate hydrolysis	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Indoxyl acetate hydrolysis	-	+	+	-	ND	-	-	ND	+	v	-	+	-	-
γ-Glutamyltransferase	ND	-	-	ND	ND	-	ND	ND	ND	ND	-	ND	-	ND
H ₂ S production (TSI)	-	-	-	-	ND	-	+	ND	-	v	+	-	-	ND

α-Haemolysis	ND	+	+	+	ND	+	-	ND	+	+	+	+	+	ND
Growth at/in/on: 18-22 °C (microaerobic)	ND	-	-	ND	ND	-	-	ND	-	-	-	ND	-	-
25 °C (microaerobic)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37 °C (microaerobic)	+	+	+	+	+	+	+	+	-	v	+	+	+	+
42 °C (microaerobic)	-	-	+	+	+	+	+	+	(-)	v	+	+	+	+
37 °C (anaerobic)	-	-	-	+	ND	-	+	ND	+	+	+	ND	-	+
37 °C (aerobic)	-	-	-	-	-	-	-	-	-	-	-	ND	-	-
CCDA	ND	+	+	ND	+	+	+	+	-	+	(+)	ND	+	ND
Glycine (1%)	-	(-)	+	-	(+)	+	(-)	+	+	v	+	(+)	+	-
Resistance to: Nalidixic acid (30 µg)	+	-	-	+	-	(+)	(+)	(+)	(+)	-	(+)	+	-	+
Cefalotin (30 µg)	+	-	+	+	+	+	-	(-)	-	-	-	-	(-)	+
H2 requirement	ND	-	-	-	ND	-	+	ND	+	+	-	ND	-	ND
S-layer present	-	-	-	-	-	-	-	-	+	-	-	-	-	-
DNA G+C content (mol%)	ND	31	30-31	36	30	29-30	36-38	29	45-46	44-46	29-33	30	32-36	29

Characteristics of reference taxa were adapted from On et al.[15] and Debruyne et al. [35,36] , (. +, 90–100% strains positive; +), 75–89% strains positive; V, 26–74% strains positive; (-), 11–25% strains positive; -, 0–10% strains positive; ND, not determined.

*Weak growth.

*** Test results differ between *C. sputorum* biovars *sputorum* (catalase and urease-negative), *paraureolyticus* (catalase-negative, urease-positive) and *fecalis* (catalase-positive, urease-negative).

These assays require approval by regulatory bodies. Some of the commercial immunoassays such as (EIAs) are available for culture-independent identification of *Campylobacter* are also used but have not been extensively validated [30-32]. In the last decade, the VIDAS/MiniVIDAS CAM (bioMérieux, Hazelwood, MO), an automated EIA for detection of thermotolerant *C. jejuni*, *C. coli* and *C. lari*, has received the most attention over [33]. With the immunological tests, more than 60 different serotypes based on somatic (O) antigen and 50 different serotypes based on heat-labile antigens (capsular and flagellar) have been identified [34-36]. For example, in United States, three immune assays have been tested by Food and Drug Administration (FDA). Each of these tests detects a common *Campylobacter* surface antigen that is shared by *C. jejuni* and *C. coli*, so the immunoassays detect both species of *Campylobacter* in stool specimens but cannot differentiate them such as: the Prospect *Campylobacter* Microplate Assay (Remel, Lenexa, KS), the Premier CAMPY (Meridian Bioscience, Cincinnati, OH), and the Immuno Card STAT CAMPY (Meridian Bioscience, Cincinnati, OH) [37].

Molecular methods have an enormous impact on the taxonomy of *Campylobacter*. Several alternative and rapid methods have been developed for detecting and confirming *Campylobacter* spp [38,39], those that include fluorescence in situ hybridization (FISH) [40], latex agglutination (commercially available; Microscreen® *Campylobacter* kit) [41]. However, the most effective confirmation

methods for identification of species are those based on the polymerase chain reaction (PCR) assays. The majority of these methods have been multiplexed and incorporated into the few real-time PCR assays, also other test such as the BAX (Dupont, Qualicon, Wilmington, DE, USA) and iQ-Check (BioRad Laboratories, Hercules, CA, USA) require an enrichment of approximately 24-48h, have been used in USA for detection of *Campylobacter* in poultry carcass with a higher contamination rate. The 16S rRNA, sequencing and DNA-DNA hybridization analysis [35,36,42], can be used for culture confirmation or for direct detection of *Campylobacter* from environmental or clinical samples. Many DNA-based methods have been also developed for molecular typing of *Campylobacter* isolates from chickens and other animal reservoirs, these include pulsed-field gel electrophoresis (PFGE) [38,43], random amplified polymorphic DNA-PCR (RAPD-PCR [44-46], and amplified fragment length polymorphism (AFLP) [47,48].

Other methods can be used for detection of *Campylobacter* such as: the whole-cell protein SDS-PAGE [35,47-50], and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry also used for identification, detection of *Campylobacter* species and related organisms in [51-53]. Sequence-based typing methods target the variable region of the fla gene (encoding the flagellin subunit), several house-keeping genes (multilocus sequence typing, MLST) (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkf* and *uncA*) [54] or the *cmp* gene (encoding the major outer

membrane protein), rpoB, groEL, hsp60 gene and cpn60 [35,55,56], have made identification of many species of *Campylobacter*. Recently the methodologies for the isolation of *Campylobacter* spp. from poultry products especially *C. jejuni* and *C. coli* are reviewed by [57].

Campylobacter: habitat & pathogenesis

Campylobacter spp., commensally organisms of poultry is known pathogens in humans and animals and has been recognized as the leading cause of bacterial gastroenteritis worldwide [58]. The *Campylobacter* are considered as zoonotic bacteria (bacteria they are transmitted from animals to humans and cause disease in humans.) and can spread easily between different animals and between animals and humans. The most prevalent subspecies about human infection are *C. jejuni* and *C. coli*, and they are typical and cause disease in humans. zoonotic pathogens. *Campylobacter* infection can present with a wide range of symptoms from watery diarrhea to dysentery, often accompanied by fever and severe abdominal cramps [1]. In humans, *Campylobacter* spp. Is one of the major causes of bacterial gastroenteritis, inducing an acute self- limiting diarrheic disease (is the unfavorable outcome of the interactions between the host, pathogen, and environments whereby conditions exist to favor pathogen growth and spread. (Disease is an abnormal state)[59]), inflammatory bowel diseases (IBD), Barrett's esophagus, and colorectal cancer [1].

Also, it's implicated in extra-intestinal infections that include bacteremia, hemolytic uremic syndrome, meningitis,

and septicemia, lung infections, brain abscesses, and reactive arthritis, in individual cases and small cohorts of patients [1,59,60]. Infection by *Campylobacter* is associated with the development of Guillain-Barre' syndrome (GBS), a neurological disorder affecting the peripheral nervous system [61,62]. More recently *C. jejuni* species has been associated with a rare form of mucosa-associated lymphoid tissue (MALT) lymphoma called immunoproliferative small intestinal disease (IPSID) [63]. Most human infections are caused by the thermophilic species *C. jejuni* or *C. coli*. *Campylobacter* causes an acute gastroenteritis characterized by fever, abdominal pain, and profuse diarrhea that is frequently bloody [64-66]. Most patients recover within 1 week without antimicrobial treatment. Bacteremia and other extraintestinal infections are uncommon complications [67]. Reactive arthritis may occur as a sequel of enteric *Campylobacter* infections in 1%-5% of patients, and Guillain-Barré syndrome occurs in approximately 0.1% of patients [68,69]. As a foodborne pathogen, the transmission of *Campylobacter* spp. To humans occurs most commonly by consumption and handling of various kinds of foods of animal origin, with poultry being the most common source [20,54]. Contact with colonized animals or drinking untreated water are potential sources of exposure

The major source of infection today is by consumption of contaminated poultry and unpasteurized milk; person-to-person transmission can occur but is uncommon (Figure 1) [70]. The contamination by these pathogenic bacteria is prevented by thoroughly cooking chicken and pasteurizing milk and water treatment.

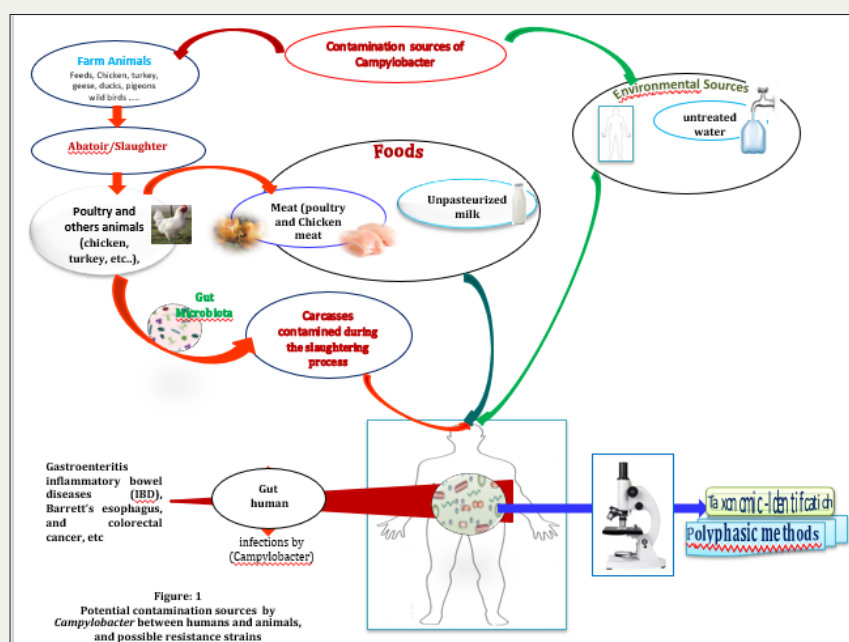


Figure 1: Potential contamination sources by *Campylobacter*.

The incubation period ranges from 1-10 days but is most commonly 2-5 days [71]. The duration of fecal shedding can range from 2-7 weeks, although the median duration of shedding is less than 3 weeks [72,73]. Otherwise, the presence of *Campylobacter* in poultry is generally varied by regions, seasons, and the production

stages and types. For example, in the United States, *Campylobacter* especially *C. jejuni* is one of the most commonly reported bacterial causes of foodborne infection [74,75] and the European Union [76]. The estimated incidence of *Campylobacteriosis* in the United States is 13.02 per 100,000 population [77], with an estimated 845,000

cases, 8,463 hospitalizations, and 76 deaths occurring annually, and with 190,566 cases of enteritis being reported in the European Union in 2008 [78].

In developed countries there is a male predisposition, a seasonal peak in cases during the late spring and summer, and a bimodal age distribution with infection being most common in children less than 1 year of age and in young adults from 15-44 years old [65]. In animal, most domestic poultry reared for consumption or egg production, including chicken, turkey, geese, ducks, pigeons, and even ostriches, as well as wild birds, are frequently infected and colonized with *Campylobacter* species and considered as natural host of these animals. Many of them (these animals) harbor *C. jejuni* in their intestinal tracts [5,79]. Many vectors are described as origin of contamination which is infected chicken carcasses and meat by *Campylobacter* and constitute a risk to consumers. The important most frequently are handling and cross contamination in the kitchen, consumption of chicken meat and during the slaughtering process [80-83]. Worldwide, an average prevalence of *Campylobacter* contamination on poultry carcasses is reported to be in the range of 60-80% [84,85].

In the past decade, a growing number of *Campylobacter* species other than *C. jejuni* and *C. coli* have been recognized as emergence humans and animal's pathogens in humans [1]. The emerging (species: a term used to describe their underappreciated roles in human and animal diseases) *Campylobacter* species are likely to contribute to the etiology of gastroenteritis, especially in cases which have no known association with other established pathogens [86-88]. *C. concisus*, *C. ureolyticus*, *C. upsaliensis*, and *C. lari*, *C. hyointestinalis*, (*C. coli*, *C. concisus*, *C. fetus*, *C. rectus* and *C. upsaliensis*) [1]. The Colonization of broiler chickens is common, and contaminated poultry meat is considered to be the most important source of human infections [82]. In association with food or water contaminated, *Campylobacters*, enter the host intestine and colonize the distal ileum and colon. Following colonization of the mucus and adhesion to intestinal cell surfaces, *Campylobacters* perturb the absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion or the production of toxin(s), or indirectly, following the initiation of an inflammatory response [89]. Many bacterial factors contribute to the colonization of *Campylobacter* in poultry [1].

These include flagella, DnaJ (heat shock protein), CiaB (*Campylobacter* invasin antigen B), PldA (phospholipase A), CadF (*Campylobacter* adhesin to fibronectin), CmeABC (multidrug efflux pump), CmeR (a pleiotropic regulator), MCP (a methyl-accepting chemotaxis protein), RpoN (sigma factor), the Kps locus (capsule biosynthesis proteins), the Pgl locus (protein glycosylation system), SOD (superoxide dismutase), Fur (ferric uptake regulator), an unnamed lipoprotein encoding gene, FucP (a fucose permease), and CbrR (a bile resistance response regulator [90]. As with other enter pathogens, motility, chemotaxis, adherence, invasion, and toxin production have been recognized as virulence properties [91]. *Campylobacter* is a mucosal-associated bacterium that has the ability to cross the mucus layer that covers the intestinal epithelium.

Some emerging *Campylobacter* species (*C. coli*, *C. concisus*, *C. fetus*, *C. rectus* and *C. upsaliensis*) have been shown to bind and invade intestinal epithelial cells. Investigations suggest that these bacteria can move through the intestinal epithelium either transcellularly (*C. fetus*) and/or paracellularly by breaking down the tight junctions associated with the barrier (*C. concisus*).

In addition, tests have shown that emerging species could release effectors proteins into epithelial cells of the host via a presumed T4SS. The S-layer induces resistance to phagocytosis and serum destruction, possibly by inhibiting the stable deposition of complement on the surface of bacterial cells during the systemic phase of infection. Emerging species can also generate a number of toxins, including tripartite cytolase distension toxin. Binding of this toxin to the surface of the host cell is provided by CdtA and CdtC, followed by delivery of CdtB into the host nucleus, which triggers cell cycle arrest and DNA damage. Other toxins include cell-bound and secreted haemolysins have the ability to lyse red blood cells (*C. coli* and *C. concisus*) [1].

Prevalence of antimicrobial-resistant *Campylobacter*

The emergence of antibiotic-resistant bacteria is a problem with significant social, medical and economic consequences. There are two distinct stages in the emergence of antibiotic-resistant bacteria: the genetic change (mutation or gene acquisition); and amplification and enrichment of resistant bacteria by exposure to antibiotics (antibiotic selection) [7]. However, antibiotic (are chemical compounds which are produced by various microorganisms (bacteria, fungi, etc.) which suppress the growth of bacteria or synthesized in laboratories. [7]) used in animals is a potential problem for human medicine because antibiotic resistant bacteria can pass through the food chain to people [7,92]. The antibiotics were first used in veterinary medicine for the treatment of mastitis in dairy cows [93]. Generally, many bacteria have evolved various resistance mechanisms to help them survive the chemical onslaught of antibiotic treatment.

These mechanisms include prevention of drug entry into the cell, as illustrated by Gram-negative bacteria

whose outer lipid layer serves as a barrier to certain antibiotics. In general, bacterial resistance to antimicrobials occurs via either mutations in chromosomal loci or acquisition of horizontally transferred mobile genetic elements such as plasmids, phages, transposons and integrons [94-97] indicate that antibiotics generally work by one of five mechanisms:

- A. Inhibition of bacterial cell wall synthesis (penicillin's, cephalosporins, bacitracin, vancomycin)
- B. Inhibition of protein synthesis (chloramphenicol, erythromycin, streptomycin, tetracyclines)
- C. Inhibition of essential metabolites (sulfanilamide and trimethoprim prevent folic acid synthesis)
- D. Injury to plasma membrane (polymyxin b, nystatin, miconazole)

E. Inhibition of nucleic acid replication and transcription (quinolones, rifampin)

Today, the use of antimicrobial agents to promote growth and control diseases in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter*. The emergence of antibiotic resistant strains demonstrates that the treatment of bacterial infections cannot rely on the use of antibiotics without some critical consideration because these can contribute to the problems with antibiotic resistance in humans [98]. Many studies have reported the prevalence of antimicrobial-resistant *Campylobacter* in animal reservoirs and poultry in different countries [59,99-103]. In the European Union report for 2012 [104] the *C. jejuni* isolates from broilers exhibit high resistance to ciprofloxacin (57.2%), nalidixic acid (55.5%) and tetracycline (40.6%), and low resistance to erythromycin (1.6%) and gentamicin (0.9%). Higher resistance was exhibited by the broiler isolates to ciprofloxacin (76.6%), nalidixic acid (70.2%) and tetracycline (74.6%), and moderate to low resistance to erythromycin (15.5%) and gentamicin (3.8%). In general in the culture-confirmed cases of *Campylobacter*, the main treatments given are macrolides [105].

The prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry was tested for their susceptibility of antibiotics in suppliers in, South Africa by Bester and Essack [106]. The result obtained show that multi resistance was detected in 23% and 43% of the isolates from broiler and layer chickens, respectively. The prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China was conducted by [107]. In this study minimal inhibitory concentrations of 11 antimicrobial agents were determined using the agar dilution method recommended by CLSI against 275 *Campylobacter*. The result obtained show that 98% of the tested *Campylobacter* isolates were resistant to quinolones and tetracyclines. The *C. jejuni* isolates exhibited a high rate of resistance to phenicol antibiotics. On the contrary, the *C. coli* isolates showed a high-level resistance to macrolides and gentamicin. The authors conclude that antimicrobial resistance is highly prevalent in the poultry *Campylobacter* isolates from China, and many of them are resistant to multiple antimicrobial agents with high MIC values. A study conducted by [108], evaluate the prevalence and antimicrobial susceptibility of *Campylobacter* spp. isolated from different chicken production systems.

The result obtained by author's show that the prevalence of resistance of *Campylobacter* isolated from all origins was 80 to 90% for the fluoroquinolones studied. Also, a high resistance of tetracycline occurrence was also found for the *Campylobacter* spp. tested (58% for *C. jejuni* and 76% for *C. coli*) [109], screened antimicrobial susceptibility of 145 *Campylobacter* strains from different origins in Italy. The result obtained by authors revealed a high level of resistance for ciprofloxacin (62.76%), tetracycline (55.86%) and nalidixic acid (55.17%). Another study by [110], investigate the prevalence of *Campylobacter* spp. In fresh chicken meat samples collected from different farms the meat of free-range

in North Western Greece and assess the respective antimicrobial susceptibility of the isolates. The prevalence of *Campylobacter* isolation was in average 28.73% and ranged from 18.51% to 50%. Therefore the prevalence reported in this study is quite close to the average prevalence of the European Union (29.6%).

Nevertheless, when comparing the reported prevalence of *Campylobacter* spp. among the Mediterranean countries (Spain, Italy, France and Greece) during 2005-2010, the results obtained are considerably lower. A high resistance rates are reported by this study in relation to ampicillin, tetracycline, ciprofloxacin and erythromycin. The practices and factors influencing the use of antibiotics in selected poultry farms in Ghana was reported by [111-113]. This study investigates the use of essential antibiotics in poultry production in 400 farms in Ghana and assesses factors influencing farmers' choice of antibiotics for use on their farms. Farmers reported the use of 35 different antimicrobial agents for management of conditions such as Newcastle, fowl pox, coccidiosis, and coryza. From these agents, 20 essential antibiotics belonging to 10 antibiotic classes were extracted. Frequently employed antibiotics were tetracyclines (24.17%), aminoglycosides (17.87%), penicillin's (16.51%) and fluoroquinolones (10.55%). Only 63% of the farms completed recommended antibiotic course durations, 58% reported following recommended withdrawal periods and 88% sought veterinary advice before administration of antibiotics. The use of antibiotic-containing agents was observed to be dependent on internal factors such as size, presence of other livestock on the farm and infections. External factors such as easy access to antibiotics also influenced farmers' use of antibiotics.

These findings call for stricter regulations on access to and use of antibiotics on poultry farms in Ghana. Another study was carried out to assess the chemicals and veterinary drugs used, and their possible occurrence as residue in poultry products in randomly selected poultry farms in Ethiopia through questionnaire and observation. The result of this study showed that antibiotics, mainly Oxytetracycline, amoxicillin, ciprofloxacin, and sulfa drugs were used in 100%, 71.4%, 28.6%, and 28.6% of poultry farms, respectively. The study also revealed that piperazine was a common anthelmintic used in 31.0% of poultry farms. The disinfectants, such as, hydrogen peroxide (83.3%), sodium hydroxide (66.7%), and formalin (19.0%) were commonly used in poultry farms though. Among the rodenticides used in farms, zinc phosphide was used more in poultry farms (33.3%). We conclude that there are high possibility of drug and chemical residues occurrence in poultry in the area. A recent study was conducted to determine the prevalence and antimicrobial resistance pattern of *Campylobacter* isolated from meat, of three different food animal species sold at retail shops in, Pakistan.

The result obtained show that from 125 *Campylobacter* isolated and tested for antimicrobial resistance against commonly used antibiotics in veterinary and human medicine, 46% isolated from chicken meat, have a highest resistance against enrofloxacin, tylosin, amoxicillin and (80%) were resistant to both ciprofloxacin

and colistin. Also, most of the isolates tested (90.4%) were resistant to more than two antibiotics and were considered as multi-drug resistant bacteria. The results obtained in this study concluded that antibiotic resistant bacteria are prevalent in animal meat in Pakistan probably due to uncontrolled use of antibiotics in food animals.

Conclusion

Campylobacter is among the major causes of food-borne illness worldwide and considered the principal cause of gastroenteritis in human. However, the contaminated food especially poultry meat by these pathogenic bacteria are considered the principal cause of an emergence humans and animal's pathogens. In poultry farms and in livestock, antibiotics are used for therapy and promotion of growth. The misuse of antibiotic is considered the most important factor selecting for emergence of antibiotic resistance. To minimize this problem, it's recommended to monitor and control bacterial infections by *Campylobacter* in food production. Antibiotics should be prudently used by the implementation a strategies and guidelines are required for limiting, controlling and minimized the spread and development of resistant bacteria and the genes that encoded for this resistance especially in poultry farms and livestock.

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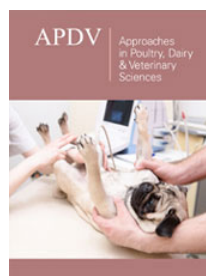
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