International Journal of Scientific Research and Engineering Development-– Volume 3 - Issue 4, July - Aug 2020

Available at www.ijsred.com

REVIEW ON CAMPYLOBACTERIOSIS: TREATMENT, SUSCEPTIBILITY TESTING AND ANTIMICROBIAL RESISTANCE

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ABSTRACT

This seminar paper is mainly prepared to review important antimicrobial drugs used to treat campylobacteriosis and mechanisms of antimicrobial resistance in campylobacter species. The disease is caused by campylobacter species which is becoming a disease of great public health concern worldwide. Antibiotics such as, macrolides (erythromycin), fluoroquinolones (ciprofloxacin, nalidixic acid) and tetracyclines are usually used in the treatment of Campylobacter infections. This pathogenic organism is increasingly resistant to antibiotics, especially fluoroquinolones and macrolides, which are the most frequently, used antimicrobials for the treatment of the disease when clinical therapy is warranted. The bacteria develop different mechanisms to resist the effect of antimicrobial agents. For example, multidrug efflux pumps like CmeABC has been identified which is giving resistance to different classes of antibiotics. The mechanism of Campylobacter's resistance to erythromycin is a point mutation in ribosomal proteins L4 and L22. Although the presence of the CmeABC efflux pump is an important co-determinant of phenotypic resistance that likely explains the high level of correlation between macrolide resistance and resistance to other antibiotics of different classes, Plasmids are responsible for the increasing level of Campylobacter resistance to tetracyclines. Antimicrobial susceptibility testing continues to play a critical role in guiding therapy and epidemiological

monitoring of resistance. As antibiotic resistance becomes increasingly prevalent in Campylobacter, the need for novel antimicrobial strategies to reduce Campylobacter in poultry and poultry products becomes more critical. Although Controlling spread of resistant bacteria by improving hygiene and infection control as well as, "One Health" approach is essential to improve the efficiency of antimicrobial resistance (AMR) research, surveillance, prevention and control systems.

Keyword: **Antimicrobials, Antimicrobial-resistance, Campylobacteriosis Campylobacter species, Susceptibility testing**

1. INTRODUCTION

Campylobacter is a zoonotic pathogen which causes food-borne enteritis with *C. jejuni* and *C. coli* being the most isolated species (Scallan *et al.,* 2011). Campylobacter species are one of the commensal bacteria in gastro-intestines of domestic animals (Butzler, 2004). They are transmitted to humans through consumption of uncooked or undercooked chicken, pork, beef, raw or unpasteurized milk, and untreated water (Shane, 2000).

Campylobacteriosis, which is caused by campylobacter species, is often self-limiting and does not require antimicrobial treatment. However, in some cases such as septicaemia or other invasive forms of the disease which characterised by severe and prolonged enteritis as well as in immune compromised or very young patients, antibacterial therapy may be needed (EFSA, 2012). However, antibiotic resistance is a significant health, social, and economic problem at this time. It leads to biological risk, which increases morbidity and mortality of animal and human (EFSA, 2008). Resistant bacteria from the intestines of food animals may be transferred to retail meat products resulting from faecal contamination during various stages of the slaughter process (Example, evisceration) and subsequent handling of animal tissue (Jackson *et al.,* 2001).

Various scientific confirmations indicate that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through food chain. This has emerged and spread resistant strains of Campylobacter,

with potentially serious effects on food safety in both veterinary and human health (Avrain *et al.,* 2003). Resistance in campylobacter species has developed to nearly all antibiotics used in veterinary medicine (Luangtongkum *et al.,* 2009). Multiple mechanisms for antibiotic resistance exist in Campylobacter (Taylor,1988). Efflux was first postulated as a mechanism of multidrug resistance in Campylobacter in 1995 (Charvalos *et al.,* 1995). In 2002, encoded multidrug resistance-nodulation-cell division (RND) efflux system was identified and characterized in *C. jejuni* (Lin *et al.,* 2002; Pumbwe and Piddock, 2002) and later in *C. coli* (Corcoran *et al.,* 2005). Since poultry are the main sources of campylobacter infection, reduction of *Campylobacter* from fresh poultry and non-poultry products may reduce the incidence of human campylobacteriosis, and development of antimicrobial resistance (Lin, 2009). The prevalence of campylobacteriosis and resistance of Campylobacter for antimicrobial agents were not summarized well recently. It is valuable to summarize the treatment options and drug resistance patterns of the bacteria. Therefore, the objectives of this seminar paper are: -

- To review important antimicrobial drugs used to treat campylobacteriosis and mechanisms of antimicrobial resistance in campylobacter species
- To describe the concepts of antimicrobial susceptibility and resistance testing

2. GENERAL REVIEW ON CAMPYLOBACTERIOSIS

Campylobacteriosis is the collective term used to describe infectious diseases caused by campylobacter's (Coker *et al.,* 2002). *Campylobacter jejuni* causes more than 90% of the infections, and 5-10% of infections are due to *C. coli* (Altekruse *et al.,* 1999; Allos, 2001). The disease is characterized by watery, non-bloody, non-inflammatory diarrhoea to severe inflammatory diarrhoea with abdominal pain and fever (Coker *et al.,* 2002). *Campylobacter* infections can also develop to Guillain-Barré syndrome (GBS), an autoimmune-mediated neurodegenerative disorder which causes acute neuromuscular paralysis (Oakland *et al.,* 2011).

A great number of environmental and animal reservoirs make the epidemiology of Campylobacter infection very complicated. A risk factor was connected with food (Siemer *et al.*, 2005) and faecal

material from livestock (Mullner *et al.,* 2010; Mohan, 2015) has been extensively studied. Urban wild birds such as ducks, goose, starlings, are contribute to enormous faecal contamination of the environment and need to be treated as an important source of campylobacteriosis for humans and farm animals (Colles *et al*., 2011). It is diagnosed by culturing the organism from stool or faecal samples (Allos, 2001).

2.1 General Characteristic of Campylobacter Species

Campylobacter microorganisms are small (0.2–0.9 μm wide and 0.2–5.0 μm long), spirally curved, and motile Gram-negative bacteria that are commonly present in the intestinal tract of domestic and wild animals (Blaser *et al*., 2008). Twenty-One *Campylobacter* species have been identified and characterized so far and among them, the most important pathogenic species being *C. jejuni* and to a lesser extent, *C. coli*. Both of these species are different from other pathogens associated with food-borne disease since they are essentially microaerophilic, able to grow in an atmosphere containing approximately 10% CO_2 and 5% O_2, at a narrow temperature range between 30°C and 46°C, and thus classified as thermophilic campylobacters (Allos, 2001). The most important species of Campylobacter are the thermotolerant (that is thrives at relatively high temperatures) species: *C. jejuni, C. coli* and *C. lari* (formerly known as "nalidixic acid resistant thermophilic Campylobacter species – NARTC"). Other species which are known to cause human illness are *C. upsaliensis, C. fetus and C. jejuni* (EFSA, 2005).

Thermotolerant Campylobacter species, especially *C. jejuni* and *C. coli*, are commonly isolated from water sources, food animals such as poultry, cattle, pigs, and sheep, as well as from cats and dogs (Jones, 2001; FAO/WHO, 2002). *Campylobacter* are motile by means of a long, single, polar, unsheathed flagellum at one or both ends of the cells. They have a characteristic rapid, darting, corkscrew-like motility although both *C. jejuni* and *C. coli* can produce non-motile variants, especially on frequent subculture (Wassenaar and Newell, 2006).

In general, all *Campylobacter*s have a low guanine and cytosine (content (29–47 mol %), reduce fumarate to succinate, are indole-negative, oxidase-positive (except

for *C. gracilis*), catalase- positive, methyl red-negative acetone-negative, reduce nitrate (except *C. jejuni* subspecies. *doyley*), hippurate negative (except *C. jejuni* subspecies *jejuni* and subspecies *doyley*) and urease- negative (except some *C. lari* strains) (Vandamme, 2000; Wassenaar and Newell, 2006). All Campylobacters are sensitive to pasteurization temperatures and all viable cells are inactivated in pasteurized or adequately cooked foods (Park, 2002).

3. IMPORTANT ANTIMICROBIAL DRUGS USED TO TREAT CAMPYLOBACTERIOSIS

Antimicrobials are drugs that kill or inhibit the growth of microorganisms or microbes. An estimated half of the globally produced antimicrobials are used for food animals (WHO, 2002). They are used in animals as therapeutics, prophylactics and growth promoters (Committee for Veterinary Medicinal Products, 1999). The most common antimicrobial agents used in the treatment of *Campylobacter* infections are macrolides, such as erythromycin, and fluoroquinolones, such as ciprofloxacin.

Tetracyclines have been suggested as an alternative choice in the treatment of clinical campylobacteriosis, but in practice they are not often used (Blaser *et al.,* 2008).

3.1 Ciprofloxacin

Ciprofloxacin is structurally a fluoroquinolone antimicrobial. It is a broad-spectrum, synthetic antimicrobial agent commonly used to treat many bacterial infections, including campylobacteriosis. Nalidixic acid, a predecessor to fluoroquinolone antimicrobials, belongs to the quinolone class of antimicrobials (Sato *et al*., 1992). Sitafloxacin is a new generation fluoroquinolone that is more effective than ciprofloxacin against *C. jejuni* strains, including those with a *GyrA* mutation (Lehtopolku *et al*., 2005). The most commonly used fluoroquinolone in animals is enrofloxacin, the main metabolite of which is ciprofloxacin (Küng *et al*., 1993).

Ciprofloxacin acts by inhibiting DNA synthesis. In a living bacterial cell, DNA is a negatively super coiled form. These negative super coils are removed by a topoisomerase II DNA gyrase, and the daughter chromosomes are separated by topoisomerase IV, allowing replication and transcription to take place (Drlica and Zhao, 1997). Quinolones act by binding these topoisomerases, and in Gram-negative bacteria the quinolones interact

primarily with gyrase and DNA, thus inhibiting super coiling and possibly forming a cytotoxic complex leading to cell death (Maxwell, 1992).

3.2 Erythromycin

Erythromycin is a macrolide antibiotic. It is the drug of choice for treating campylobacteriosis, because of it is a broad-spectrum, fairly inexpensive and well-tolerated antibiotic that is effective against *Campylobacter* species when administered during the first days of illness (Salazar-Lindo *et al.*, 1986).

The mode of action of erythromycin is inhibition of protein synthesis by binding to the 23S rRNA in the 50S ribosomal subunit (Schlunzen *et al.*, 2001). This sterically hinders the binding of the peptidyl transfer RNA, which results in inhibition of translocation, thus halting the elongation of the developing peptide chain (Brisson-Noel *et al.*, 1988).

3.3 Tetracycline

Tetracycline acts by binding to discrete sites on the ribosomal 30S subunit (Chopra *et al.*, 1992). Its primary antimicrobial effect takes place by direct steric hindrance by binding to the A site in the 30S subunit, thus hindering the movement of transfer RNA (Harms *et al.*, 2003).

4. MECHANISM OF ANTIMICROBIAL RESISTANCE

Campylobacter can acquire antibiotic resistance by spontaneous mutations and horizontal gene transfer via natural transformation, transduction, and conjugation (Kumar *et al.,* 2016). For example, the presence of conjugative plasmids containing *tetO*, have substantial roles in disseminating tetracycline resistance in *Campylobacter.* Of the known antibiotic resistance determinants in *Campylobacter*, CmeABC is the best characterized (Pérez-Boto *et al.,* 2014). An increasing numbers of *Campylobacter* isolates have developed resistance to fluoroquinolones and other antimicrobials such as macrolides, tetracycline, aminoglycosides, and betalactams (Table 1). Furthermore, intrinsic resistance in *C. jejuni* and *C. coli* has been described against penicillin and most of the cephalosporins as well as trimethoprim, sulfamethoxazole, rifampicin, and vancomycin (Fitzgerald *et al*., 2008).

Table 1. Antibiotic resistance mechanisms of Campylobacter

Antibiotic class	Resistce mechaims
Aminoglycoside	Modification of the antibiotic by aminoglycoside-modifying enzymes. Contribution of efflux is not clear
Beta-Lactam	Enzymatic inactivation of the antibiotic by β-lactamase (penicillinase,) Decreased membrane permeability of most anionic and Mw > 360 kDa antibiotics due to MOMP. efflux through CmeABC and possibly others
Fluoroquinolone	Modification of the DNA gyrase target (Thr-86-ile; also Asp-90-Asn, Ala-70-Thr). efflux through CmeABC
Macrolide	Mutations in 23S rRNA . Contribution of mutations in ribosomal proteins L4/L22 is likely minor. efflux through CmeABC and possibly others Decreased membrane permeability due to MOMP
Tetracycline	Modification of the target ribosomal A site by TetO binding. efflux through CmeABC and possibly others Contribution of decreased membrane permeability due to MOMP is not clear

(***Source: Yuli et al., 2016).***

4.1 Resistance to Fluoroquinolones

Resistance to the fluoroquinolones is mainly due to amino acid(s) substitution(s) in the quinolone resistance-determining region (QRDR) of the corresponding topoisomerase. QRDR is located within the DNA-binding domain on the surface of these enzymes. There are several different single *GyrA* modifications reported to be associated with fluoroquinolone resistance in *Campylobacter* species: Thr86Ile, Asp90Asn, Thr86Lys, Thr86Ala, Thr86Val, and Asp90Tyr. However, the most frequently observed mutation in quinolone resistant *Campylobacter* is the C257T change in the *gyrA* gene, which leads to the Thr86Ile substitution in the gyrase and confers the high-level resistance to this group of antimicrobials (Payot *et al.,* 2006).

The CmeABC multidrug efflux pump is another most common efflux system in *Campylobacter* and works in synergy with GyrA mutations in causing fluoroquinolone resistance (Luo *et al.*, 2003). Inactivation of the CmeABC efflux pump by insertional inactivation of *cmeB* or with efflux pump inhibitors leads to increased susceptibility to different antibiotics, including those to which *Campylobacter* are intrinsically resistant, showing that CmeABC plays a key role in both intrinsic and acquired resistance of *Campylobacter (*Akiba *et al.,* 2006*).*

4.2 Resistance to Macrolides

The main mechanisms of resistance to macrolides in Campylobacter are target modification, efflux and altered membrane permeability. The first two mechanisms act synergistically to confer high- level macrolide resistance (Cagliero *et al*., 2006).

Mutations in 23S rRNA block the interaction of macrolide drugs with the 50S ribosomal subunit, and thus, confer macrolide resistance. Specifically,

mutations at base positions 2074 and 2075 are associated with high-level macrolide resistance in *C. Jejuni* and *C. coli* (Mamelli *et al.*, 2005). Mutations at these positions are likely to block the binding of macrolides to their inhibitory site on the 23S ribosomal subunit (Pfister *et al.*, 2004). The predominant mutation in erythromycin resistant strains is A2075G (Gibreel and Taylor, 2006), and in some strains these two mutations have been found to co-exist (Vacher *et al.*, 2003; Vacher *et al.*, 2005). The chromosome of *C. jejuni* and *C. coli* contains three copies of the 23S rRNA gene (Fouts *et al.*, 2005). In addition to 23S RNA mutations, mutations in ribosomal proteins L4 (G74D) and L22 (insertions at position 86 or 98) have been implicated in conferring macrolide resistance to *C. jejuni* and *C. coli* (Cagliero *et al.*, 2006).

4.3 Resistance to Tetracyclines

Tetracycline resistance in *C. jejuni* and *C. coli* involves ribosomal protection and efflux mechanisms. The ribosomal protection is mediated by a protection protein termed Tet(O) (Manavathu *et al.*, 1988) that is structurally related to translational ribosome-binding proteins (Connell *et al.*, 2003). Tet(O) is coded by a gene located on plasmids of different sizes and in some strains chromosomally (Lee *et al.*, 1994; Gibreel *et al.*, 2004).

Several studies have demonstrated that *tet(O)*-carrying plasmids can be transferred via conjugation between *C. jejuni* and *C. coli* strains *in vivo* and *in vitro.* Tet(O) acts by removing tetracycline bound to the A-site in the ribosomal 30S subunit (Pratt and Korolik, 2005). Specifically, Tet(O) induces a conformational change, which is functionally distorts the tetracycline binding site, resulting in the release of tetracycline. Even after the Tet(O) has left the ribosome, the induced conformation persists, thus preventing rebinding of the tetracycline (Connell *et al.*, 2003).

4.4 Resistance to Other Antimicrobial Agents

Aminoglycosides are protein synthesis inhibitors of many Gram-positive and gram-negative organisms. They are act by binding to the decoding region in the A-site of the ribosomal 30Ssubunit. This interaction results in aberrant proteins by interfering with accurate codon- anticodon recognition and in disruption of elongation of nascent proteins by inhibiting the translocation of tRNA from the A-site to the P-site (Jana and Deb, 2006). Aminoglycoside resistance is mediated by

enzymatic modification that diminishes affinity of aminoglycosides for the rRNA A-site (Llano-Sotelo *et al.*, 2002). These enzymes fall into three classes: aminoglycoside acetyltransferases, aminoglycoside adenyl transferases and aminoglycoside phosphotransferases, each of which has its own characteristic modification sites and substrates (Engberg *et al*., 2006). The three mechanisms that are mediate β-lactam resistance in Campylobacter are enzymatic inactivation by chromosomally encoded β-lactamases, reduced uptake due to alterations in outer membrane porins and efflux (Lachance *et al.,* 1991).

5. MULTIPLE DRUG RESISTANCE IN CAMPYLOBACTER SPECIES

According to definition, a bacterial strain can be considered resistant to a certain antimicrobial agent if it is able to propagate in higher concentrations in that agent than other strains of the same species or genus. Multiple drug resistance (MDR) in bacteria is associated with efflux systems and structural components of the cell membrane. These mechanisms control the transport of structurally unrelated substances in and out of the cell (Guardabassi and Courvalin, 2006).

5.1. *Efflux Systems and Efflux Pump Effectors*

Energy-dependent drug efflux pumps are used in bacteria for extruding metabolites and toxic compounds, including antimicrobial agents (Li and Nikaido, 2004). In *C. jejuni*, several putative efflux systems have been identified (Parkhill *et al.*, 2000; Ge *et al.*, 2005). Of these, two systems have been characterized as conferring MDR resistance, namely CmeABC and CmeDEF (Pumbwe *et al.*, 2005). Homologous CmeABC system has also been characterized in *C. coli* (Corcoran *et al.*, 2005) and identified in *C. lari*, *C. upsaliensis* and *C. fetus* (Guo *et al.*, 2010).

CmeABC is coded by an operon consisting of three genes, *cmeA*, *cmeB* and *cmeC,* which code for a periplasmic fusion protein, an inner membrane drug transporter and an outer membrane protein, respectively (Lin *et al.*, 2002). CmeB belongs to the resistance nodulation cell division (RND) family of efflux transporters (Pumbwe and Piddock, 2002).

Since efflux pump systems have a key role in the antimicrobial resistance of *C. jejuni* and *C. coli*, inhibition of efflux would provide an important means for preventing emerging resistance and multidrug resistance in bacteria. To achieve this

inhibition, synthetic and natural compound libraries have been screened with the aim of finding substances capable of reversing the action of the efflux pumps (Lin *et al.*, 2002; Corcoran *et al.*, 2005).

5.2 Role of the Membrane Proteins in Campylobacter Resistance

Drugs can cross the bacterial membrane and enter the cell by using porin channels or by diffusing through the lipid bilayer. Porin channels are postulated to be mainly used by relatively small drugs, such as β-lactams, tetracycline, chloramphenicol and fluoroquinolones, whereas large molecules

such as macrolides, diffuse slowly across the lipid bilayer (Nikaido, 2003). In *Campylobacter*, two outer membrane porins have been characterized: in *C. jejuni* and *C. coli,* the major outer membrane porin (MOMP) protein, encoded by *porA*, and in *C. jejuni* Omp50 (Bolla *et al.*, 2000). Very limited functional information is available about these porins, and thus far, no evidence has emerged that they play any role in MDR of *Campylobacter* (Pumbwe *et al.*, 2004).

6. SPECIES SPECIFIC ANTIMICROBIAL RESISTANCE

The PCR method with specific primers are used for the purpose of identification of colonies as *C. jejuni* or *C. coli* (On ST *et al.,*2003). According to the results of numerous studies a significant rise in resistance to fluoroquinolones, tetracycline, and erythromycin has been demonstrated in *C. jejuni* and *C. coli* isolates from various sources such as humans, animals and food (Table 2) (Rozynek *et al.,* 2008).

Table 2. Species-specific resistance profile of strains from food animals

Antibiotics	***C. jejuni***	***C. coli***	***C. lari***
	Resistance (%)	**Resistance (%)**	**Resistance (%)**
Nalidixic acid	23	2.9	100
Cefotaxime	92.6	66.7	93.3
Erythromycin	99.5	100	100
Tetracycline	69.8	89.8	96.7
Kanamycin	17.1	8.7	36.7
Gentamicin	21.1	1.4	26.7
Ampicillin	96.5	84	96.7
Imipenem	0	0	0
Cephalexin	99.5	72.5	100
Ciprofloxacin	40.2	37.7	93.3
Chloramphenicol	87.7	43.5	66.7
Norfloxacin	25.5	2.9	83.3

(Source: Akosua et al., 2017)

7. PREVALENCEAND ANTIMICROBIAL RESISTANCE

The prevalence of Campylobacter species in chicken meat, chevon and milk samples were observed 17.33%, 6% and 0%, respectively (Singh *et al.,*2009). Antimicrobial resistance in campylobacters isolated from foods of animal origin appears to be increasing in many countries. There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Looveren van *et al.,*2001).

Molecular detection of antibiotic resistance genes has demonstrated that identical elements were found in bacteria that colonize both animals and humans. This suggests that bacteria originating from food of animal origin aid in the spread of

resistant bacteria and resistance genes from animals to humans via the food chain (Jideani and Aiyegoro, 2013). Molecular typing methods, such as multilocus sequence typing and pulsed field gel electrophoresis, revealed that certain genotypes of *C. jejuni* from ruminants are indistinguishable from human isolates (Stanley and Jones, 2003).

Campylobacter develop resistance to antibiotics like fluoroquinolones and macrolides by mutations. Besides spontaneous mutations, they are also able to acquire resistance determinants by natural transformation, transduction or conjugation, example, conjugation of tet(O)-carrying plasmids (Zhang and Plummer, 2008).

Development and transmission of antibiotic resistant Campylobacter is complicated by the fact that Campylobacter is a zoonotic pathogen and is exposed to antibiotics used in both animal production and human medicine. Evidence suggests that the most important factor contributing to the rapid emergence of antibiotic-resistant *Campylobacter* strains in the food chain is the use of antibiotics, mainly the fluoroquinolones, as growth promoters, therapeutics and prophylactics in food animals (Alfredson and Korolik, 2007).However, continuous use of macrolides in food- producing animals at subtherapeutic concentrations is a major risk factor influencing the emergence of erythromycin resistance of Campylobacter isolated from animals and environmental sources (Rozynek *et al.,* 2013).

8. ANTIMICROBIAL SUSCEPTIBILITY TESTS

Antimicrobial susceptibility testing continues to play a critical role in guiding therapy and epidemiological monitoring of resistance. There are several methods to determine in vitro susceptibility profile(s) of *Campylobacter* to a range of antibiotics, including disc diffusion, broth microdilution, agar dilution and the Epsilometer-test (E-test) (Aarestrup *et al.,* 2008). Recently, the agar dilution method has been considered a standard antimicrobial susceptibility testing method for thermophilic *Campylobacter* species (McDermott *et al*., 2004). Although the agar dilution method is reliable and highly reproducible and also provides quantitative MICs, it is a labour-intensive, time-consuming, and costly test (Caprioli *et al*., 2000). Alternatively, the agar diffusion test, such as the disk diffusion method, is simple and inexpensive and can provide reproducible results if it is conducted carefully with appropriate standardization and quality controls (Potz *et al.,* 2004).

In vitro antimicrobial susceptibility testing involves measuring the antimicrobials activity against the microorganism by determining the MIC or inhibition zone. The MIC was defined as the lowest concentration of the antimicrobial agent that completely inhibited the visible growth on the plates (Moore *et al.,* 2005; Gaudreau *et al.,* 2007).

Table 3. Antibiotic resistant *C. jejuni* isolates recovered from humans, retail meats, and chicken carcasses

Antibiotic	Humans	Chicken Breasts	Ground Turkey	Chicken Carcasses
Erythromycin	6 (0.8)	4 (0.9)	0	**1 (0.4)**
Azithromycin	6 (0.8	4 (0.9)	0	**1 (0.4)**
Clindamycin	7 (1.0)	3 (0.7)	0	**0**
Gentamicin	0	0	0	**0**
Tetracycline	336 (47.4)	201 (47.2	9 (75.0)	**128 (56.1)**
Ciprofloxacin;	**138 (19.5)**	**71 (16.7**	**6 (50.0)**	**20 (8.8)**

(Source: FDA, 2010)

In Ethiopia there are a few studies on antimicrobial susceptibility of Campylobacter. Forty-three *Campylobacter jejuni isolated* were

investigated in order to estimate antimicrobial resistance of Campylobacter species recovered from different districts located at Debrebirhan Ethiopia by Chanyalew and Asrat (2016) and they found that the highest level of resistance of *Campylobacter* was to cephalotin followed by ampicillin, tetracycline and erythromycin.

Table 4. Antimicrobial susceptibility of *C. jejuni* isolated from sheep at Debre-birhan Ethiopia

Antibiotic	Number (percentage)		
	Susceptible	Intermediate	Resistance
Ampicillin	28(65.1)	-	15 (34.9)
Erythromycin	39 (90.7)	1 (2.3)	3 (7)
Cephalothin	-	-	43 (100)
Nalidixic acid	43 (100)	-	-
Penicillin	43 (100)	-	-
Streptomycin	41 (95.3)	-	2 (4.7)
Tetracycline	32 (74.4)	1 (2.3)	10 (23.3)
Gentamicin	42 (97.7)	-	1 (2.3)
Ciprofloxacin	42 (97.7)	-	1 (2.3)
Chloramphenicol	41 (95.3)	-	2 (4.7)

(Source: Chanyalew and Asrat, 2016)

9. TREATMENT AND REDUCING CAMPYLOBACTER COLONIZATION

As antibiotic resistance becomes increasingly prevalent in *Campylobacter*, the need for novel antimicrobial strategies to reduce *Campylobacter* in poultry and poultry products becomes more critical. This is primarily driven by the need to reduce the economic and human health burden incurred by antibiotic-resistant campylobacteriosis (Duarte *et al.,* 2016).

9.1 Anticampylobacter Compounds

Recently, study was conducted to identify small molecule inhibitors of *Campylobacter* flagellar expression known colonization factor. Screening a library of approximately 147,000 small molecules, the authors identified compounds that modestly inhibited flagellar motility and several other compounds, termed 'campynexins,' that inhibited *Campylobacter* growth *in vitro.*

The molecules of greatest interest are those that specifically exhibit activity toward *Campylobacter* – to minimize the effects on beneficial microbes in the gastrointestinal tract – and demonstrate efficacy *in vivo* .The anti-*Campylobacter* molecules described todays are belong to five chemical classes that have been established as antimicrobial: aryl amines, piperazines, pyridiazinones, sulphonamides, and piperidines (Johnson *et al*., 2015)

9.2 Probiotics

The probiotic genera that are most commonly evaluated for their ability to reduce *C. Jejuni* colonization are *Lactobacillus, Bacillus*, and *Enterococcus*, as these are well characterized and commonly found in the intestines of animals (Arsi *et al.,* 2015; Thomrongsuwannakij *et al.*, 2016). Researchers have also investigated the efficacy of *Bifidobacterium* species and *Saccharomyces cerevisiae* at inhibiting *C. jejuni* colonization and growth (Bratz *et al.,* 2015; Fanelli *et al.,* 2015). It was observed that *L. helveticus* adhered to the epithelial cells, suggesting that competitive exclusion may have contributed to the reduction in *C. jejuni* invasion (Wine *et al.,* 2009).

The mechanisms of competitive exclusion, includes the occupation of adhesions sites and receptors, secretion of antimicrobial substances, and competition for essential nutrients. Another study reported that multiple *Lactobacillus* strains inhibited the growth of *C. jejuni in vitro* due to organic acid production by these microorganisms (Bratz *et al.,* 2015). *Lactobacillus* species lower pH to create a more hospitable environment for themselves, an effect that is increased when multiple strains are present (Wooten *et al.,* 2016).

9.3 Campylobacter Bacteriophage

Bacteriophages are viral predators of bacteria that are ubiquitous in the environment and often exhibit exquisite specificity against their host bacterial species. Bacteriophages could potentially be used without impacting the normal microbiota of the host and may be suitable for reducing *C. jejuni* colonization at the farm level, thus decreasing transmission to the food chain. These attributes make bacteriophages an attractive anti-*Campylobacter* treatment. Bacteriophages that are effective against *Campylobacter* have been isolated from multiple sources, including sewage, pig manure, poultry carcasses, and broiler chickens (El-Shibiny *et al.,* 2009).

9.4 Campylobacter Vaccine for Poultry and Humans

Vaccination of poultry against *Campylobacter* could eliminate the microorganism from birds and reduce the incidence of human campylobacteriosis in the developed world (Avci, 2016). However, the cost of campylobacteriosis to public health systems and the loss of labour productivity is substantial, therefore the main rationale for developing a

Campylobacter vaccine would be to reduce potential human health risks, enhance food safety, and decrease the high costs associated with the disease. For the reasons described above, the need for a *Campylobacter* vaccine may not be driven by the market itself but will likely require intervention by government agencies (Lund and Jensen, 2016). Despite the substantial amount of research directed toward vaccine development, currently there is no vaccine on the market to reduce *Campylobacter* loads in the gastrointestinal tract of chickens (Meunier *et al.,* 2016).

10. CONCLUSION AND RECOMMENDATION

Campylobacter is the most common cause of bacterial gastroenteritis in the world. It can cause a gastrointestinal infection called campylobacteriosis, in both human and animals. Antibiotics, such as macrolides, quinolones and tetracycline are used to treat gastrointestinal *Campylobacter* infections. Campylobacter resistance to a particular antimicrobial is often a result of a mutation in the gene encoding the target site of that antimicrobial. These target sites, such as DNA gyrase and RNA polymerase, are essential for bacterial propagation, and mutations encoding these sites may result in reduced fitness of antimicrobials.

The indiscriminate use of antibiotics in the human population as well as the use of antibiotics in animal husbandry for treatment, growth promotion and off-label uses have led to an increase in antibiotic-resistant in Campylobacter infections. In general, mechanisms of antibiotic resistance in campylobacter species include: -

1) Modification of the antibiotic's target and/or its expression (Example: DNA gyrase mutations)
2) Inability of the antibiotic to reach its target (that is expression of the major outer membrane protein or MOMP)
3) Efflux of the antibiotic (that is., multidrug efflux pumps such as CmeABC)
4) Modification or inactivation of the antibiotic (example: β-lactamase production)

The phenotypic test methods to test the susceptibility and resistance of pathogens to a particular antibiotic, include diffusion (disk and E-test) and dilution (broth and agar dilution). The current status of present and proposed treatments to combat *Campylobacter* infection in humans and colonization in animal reservoirs are includes, treatment by anti-*Campylobacter* compounds, probiotics, bacteriophage and vaccines all of which may be successful at reducing the incidence of campylobacteriosis in

humans and/or colonization loads in poultry. Therefore, an understanding of the antibiotic resistance mechanisms in *Campylobacter species* is needed to provide proper therapy both to the veterinary and human populations.

Based on the above conclusion the following points are recommended;

- The foods of animal origin are source of Campylobacter infections to human beings. Thus, the development of antibiotic-resistant strains emphasizes the requirement of better surveillance and monitoring of the foods of animal origin and the use of antimicrobials in veterinary and human medicine require careful regulation
- Restrictions on the use of certain antimicrobial classes, for example; fluoroquinolones as growth promoters.
- Controlling spread of resistant bacteria by improving hygiene and infection control
- A "One Health" approach is essential to improve the efficiency of AMR research, surveillance, prevention and control systems.

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