Multidrug efflux pumps in zoonotic foodborne pathogen *C. jejuni*

College of veterinary medicine University of Al-Qadisiyah

A study submitted by

Mushtaq Majid Jebur

Under supervision of Dr. Dhama Alsallami

June 2021


وقل
رب زدني علما

Dedication

To the owner of mercy on the face of the earth

To from heaven under her feet ...

to my mother

To whom was the bond and appointed in every step I took.

To my father

To whom my supervisor is proud of

(lecturer Dhama Kadim)

To my colleagues and colleagues .....

Accept the soul and fulfil its virtues

You are with the soul, not in the body, a person.

researcher

إلى صاحب الرحمة على وجه الأرض

... من السماء تحت قدميها

إلى أمي

.لمن كان السند والمعين في كل خطوة قمت بها

إلى والدي

لمن يفتخر مشرفي

..... لزملائي وزملائي

تقبل الروح وتحقق فضائلها

.أنت مع الروح، لا في الجسد، مع الإنسان

الباحث

Abstract

C. jejuni is a widespread foodborne pathogen. It considered as the main cause of human enteritis in industrialized countries. Poultry like chicken and birds are the source of infections in humans, causing mild to severe abdominal cramps and watery or bloody diarrhea with fever. However, the information that available about *C. jejuni* biology and pathogenicity still less than other pathogens and it yet less common. The growing knowledge about *C. jejuni* value as a dangerous pathogen, as well as the availability of new model systems, genetic and genomic technologies helped in develop the interest researches about Campylobacter spp. recently.

Pathogens including Campylobacter spp. are improved their strategies to resist the antibiotics through various mechanisms. One of the effective strategies is extrusion of antibiotics outside the bacterial cell by specific proteins known as efflux pump transporters. Also, the incorrect use of antibiotics in medication was the main factor that caused development of the multidrug resistant (MDR) pathogens. In addition, some pathogens are naturally resistant to certain antibiotics due to specific genes they have inside their genome. So, in this review the focusing was on role of the efflux pump systems in *C. jejuni* strains and their effect to increase surviving of this pathogen inside the host.

Campylobacter pathogen

C. jejuni belongs to the genus of bacteria and is among the most common causes of bacterial infection in humans worldwide. It is one of the most important species from microbiology and public health perspective. *C. jejuni* commonly associated with poultry, and is mostly found in animal faeces. Campylobacter genus is a spore-shaped, non-spore-negative, micro-moisture-loving, non-fermented bacterium with a single flagellum at one pole (Balaban and Hendrixson, 2011). It is oxidase positive and grows at 37 - 42 ° C (Ryan *et al*., 2004). *C. jejuni* is microaerophilic, and thus requires oxygen at sub-atmospheric levels to live and grow. In general, strains of *C. jejuni* grow in an atmosphere with between 2 to 13 % oxygen (Neill *et al*., 1985). It cannot persist in the high oxygen concentrations of the atmosphere but is also unable to grow without at least some oxygen for respiration and synthesis of nucleic acids (Krieg and Hoffman, 1986; Kelly *et al*., 2001).

When exposed to oxygen in the atmosphere, *C. jejuni* has the ability to transform into a coccoid form as a survival strategy. This type of pathogenic bacteria is one of the most common causes of human gastroenteritis in the world. Food poisoning caused by Campylobacter spp. can be extremely devastating (Crushell *et al*., 2004). It has been linked to the later development of Guillain-Barré syndrome (GBS), which usually develops within (2-3) weeks of the initial illness (Fujimoto and Amako,1990). Individuals with newly infected develop Guillain-Barré syndrome at a rate of 0.3 per 1,000 infections (Fujimoto and Amako, 1990). Reactive arthritis is also associated with a chronic condition of *C. jejuni* infection (McCarthy and Giesecke, 2001).

C. jejuni is naturally eligible for genetic mutation. Which is a sexual process that involves transferring DNA from one bacterium to another through an interfering medium, and integrating the donor's sequence into the recipient's genome by homologous recombination. For example, it freely takes the foreign DNA that contains genetic information responsible

for antibiotic resistance. Antibiotic resistance genes are transferred more frequently in biofilms than are transferred between planktonic cells (Bae and Jeon, 2014). Basically, *C. jejuni* has been recognized all through the world as a common cause of bacterial diarrhea (Butzler and Skirrow, 1979; Karmali and Fleming, 1979). The life form is microaerophilic, and so requires extraordinary conditions for determination and development. Strategies created by Butzler and colleagues in Belgium, and Skirrow within the joined together Kingdom have empowered numerous microbiological research facilities to confine this life form stools and have driven to its acknowledgment as a critical enteric pathogen (Butzler *et al*., 1978; Butzler and Skirrow, 1979). *C. jejuni* lacks a type III secretion system, common in many intestinal pathogenic bacteria, to secrete and transmit proteins that affect host cells. So, it uses a flagellar export apparatus to secrete proteins into host cells (Konkel *et al*., 2004).

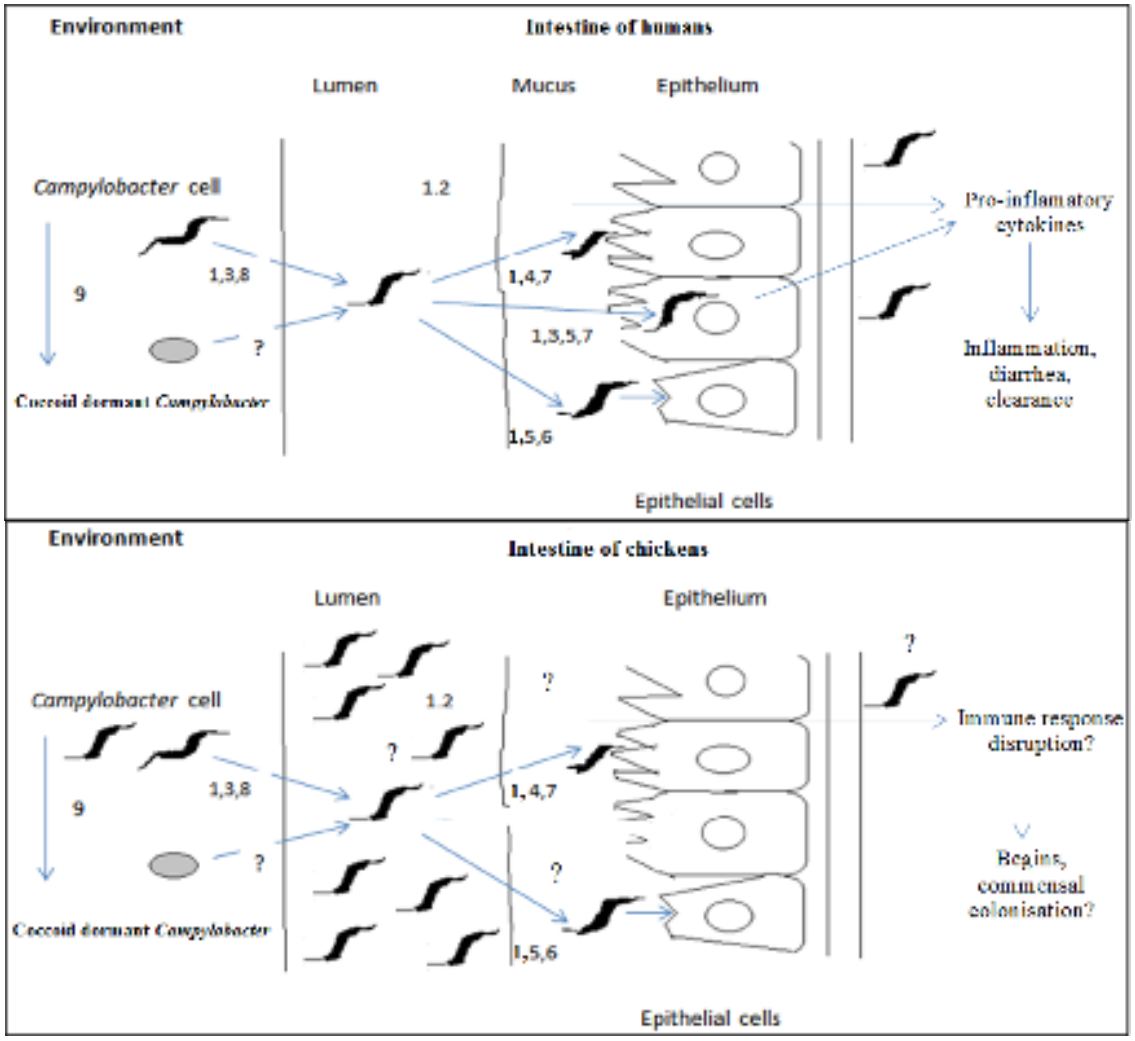

Fig.1: Stages of *C. jejuni* colonisation in human and chicken intestines. *C. jejuni* cells pass the mucus layer and attach to the epithelial cells of the intestine. Adapted from van Vliet and Ketley (2001) and Young *et al*. (2007).

C. jejuni pathogenesis and routs of transmission

Pathogenesis is the process by which disease develop. Both host and pathogen-specific factors play a role in the pathogenesis of *C. jejuni* infection. Clinical outcome after infection is influenced by the host's health and age (Tauxe *et al*.,1992). As well as *C. jejuni*-specific humoral immunity from previous exposure Blaser (Sazie and Williams ,1987). *C. jejuni* infection occurred after ingestion of as few as 800 bacteria in a volunteer study (Black *et al*., 1988). Infection rates rose as the dosage was increased. When inoculum was consumed in a suspension buffered to minimize gastric acidity, the rate of illness tended to increase (Black *et al*., 1988). Chemotaxis, motility, and flagella are important virulence factors because they are necessary for attachment and colonization of *C. jejuni* in the gut epithelium (Ketley, 1997).

C. jejuni infection appears as a heterogeneous syndrome with a wide range of clinical, immunological and pathophysiological findings (Asbury and McKhann,1997). A variety of infectious agents have been suggested as a possible triggering factor in Guillain-Barré syndrome (GBS) patients. In the United Kingdom and the Netherlands, *C. jejuni* infection was identified as the dominant infection and cytomegalovirus (CMV) as the second most prevalent (Jacobs and Rothbarth, 1998).

Other infections associated with GBS were Epstein-Barr virus (EBV) and mycoplasma pneumonia (Schessl *et al*., 2006). A recent multicentre study in children with GBS showed that coxsackieviruses were associated with previous infections (Schessl *et al*., 2006). The relative frequency of previous infections may vary between countries. In northern China, *C. jejuni* infection occurs in up to 66% when contaminated food or water is ingested, and the infectious dose can reach 800 bacterial cells (Kaakoush *et al*., 2015).

To initiate an infection, the pathogen needs to penetrate the intestinal mucosa of the host, *C. jejuni* is using its high mobility and spiral shape for that. The bacteria must then attach to the

intestinal gut cells and once attached they can cause diarrhea by releasing toxins. *C. jejuni* bacteria release many different types of toxins that vary from strain to strain, especially enterotoxins and cytotoxins, and these toxins are strongly associated with enteritis (Biomed, 1994). Iron acquisition, host cell invasion, toxin production, inflammation and active secretion, and epithelial disruption with serial fluid leakage are all possible virulence determinants once colonization has been occurred (Ketley, 1997).

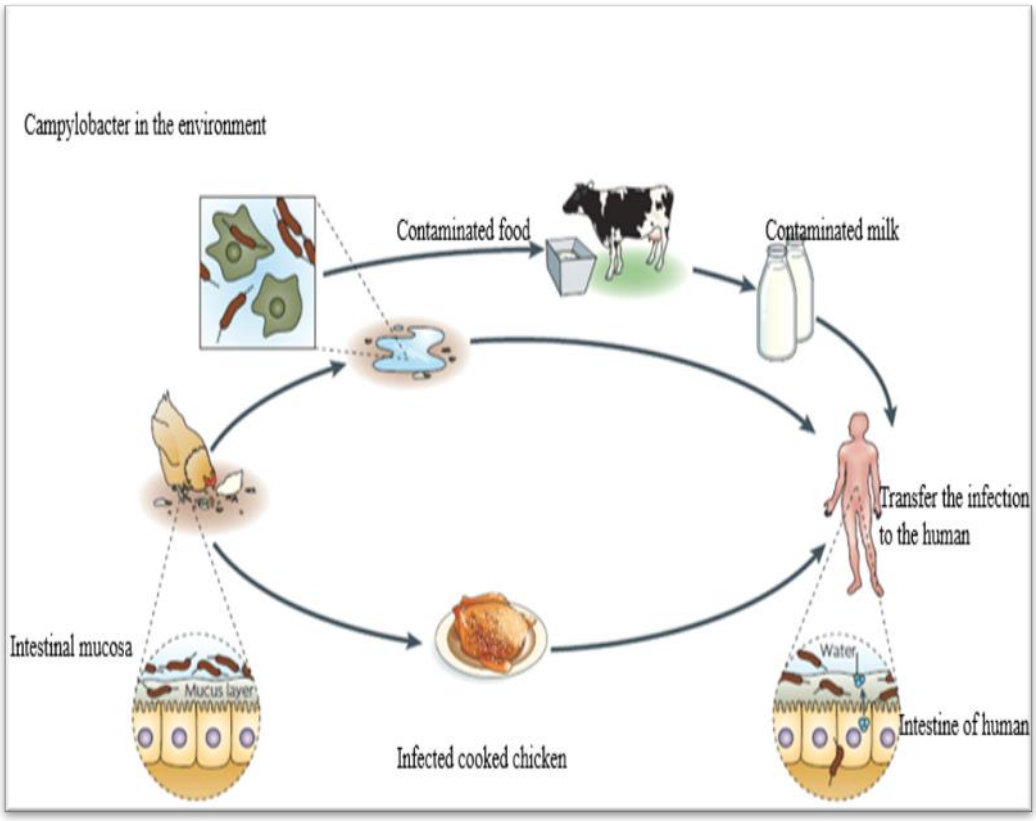

Fig. 2: Sources of *Campylobacter jejuni* infection. Environmental reservoir can lead to human infection by *C. jejuni* by colonisation of the intestinal mucosa and transmission through the faecal-oral route within chicken flocks. *C. jejuni* can enter water sources to cause infection to humans directly or indirectly, and by consumption of infected milk or meat. From Young *et al*. (2007).

Efflux pumps in Gram- negative bacteria

Since increase the pathogenic bacterial strains that able to resist different classes of antibiotics. Multidrug resistant MDR bacteria are turning in to an actual warning for public health worldwide (Mosolygó *et al*., 2019; Rao *et al*., 2018; Munita and Arias, 2016). The random use of antibiotics in medications caused increase the resistance of bacteria to various antibiotics. Such as methicillin resistance property in *Staphylococcus aureus* ،vancomycin resistance in *Enterococcus faecalis*and and Fluoroquinolone in *C. jejuni*. Also, bacteria may be naturally resistant to some antibiotics as specific genes carried on plasmid or in their own genomic DNA like tetracycline resistance in *C. jejuni* (Iovine, 2013; Levy, 1998; Okeke *et al*., 2005; Kumar and Varela, 2013).

Efflux pump systems are existing in both Gram positive and Gram-negative pathogens. For example, in *E. coli* the genome has around 39 efflux pumps that recognized and about 31 in *S. aureus* (Paulsen *et al*., 1998; Schindler *et al*., 2015). Efflux pumps are more than only antibiotic resistant factors. They even help the microorganisms to pump out the heavy metals, solvents and biocides or any harmful agent (Blanco *et al*., 2016). Efflux of antimicrobial substances outside the bacterial cell considered as a key for multidrug resistant MDR in both Gram positive and negative bacteria. Also, efflux pump is one of the important and old strategies used by pathogens to exclude the harm materials like heavy metals, dyes, antibiotics (Amaral *et al*., 2014).

Gram-negative microbes express a plenty of efflux pumps that are competent of transporting basically shifted particles, counting anti-microbials out of the bacterial cell. These effluxes bring down the intracellular anti-microbial concentration, permitting microbes to outlive at higher anti-microbial concentrations. Overexpression of a few efflux pumps can cause clinically pertinent levels of anti-microbial resistance in Gram-negative pathogens (Blair *et al*., 2014). These effluxes also cause decrease concentration of antibiotics inside the bacterial

cell and enable bacteria to survive at higher antibiotic concentrations. Clinically, overexpression of some efflux pumps resulted in a relevant level of antibiotic resistance in Gram-negative pathogens (Blair *et al*., 2014).

Contaminations caused by Gram-negative microbes are troublesome to treat since they are inherently resistant to numerous anti-microbials. This originates before the utilize of anti-microbials in individuals and creatures, and is autonomous of even quality exchange and unconstrained transformations (Cox and Wright, 2013). The double-membrane structure of Gram-negative microscopic organisms and the natural generation of efflux pumps permits anti-microbials to be sent out, in this way decreasing intracellular concentrations (Nikaido, 2001).

Gram-negative microscopic organisms have various efflux pumps in their films; these transport a wide assortment of particles out of the bacterial cell. A few of these pumps can transport anti-microbials. Pumping anti-microbials out of the bacterium diminishes the medicate concentration interior the cell, permitting microscopic organisms to outlive at higher outside concentrations of antimicrobial sedate, driving to survival and resistance. However, a few efflux pumps are able to transport anti-microbials of more than one basic course, in this manner conferring multidrug resistance (MDR) (Saier *et al*., 1998). In addition, efflux pumps are essential to the physiology of the life form and a few have parts other than conferring resistance to antimicrobials. For example, RND efflux pumps of Gram-negative microbes are required to remove the harm materials (Bina *et al*., 2008) and biofilm arrangement (Baugh *et al*., 2012; Kvist *et al*., 2008).

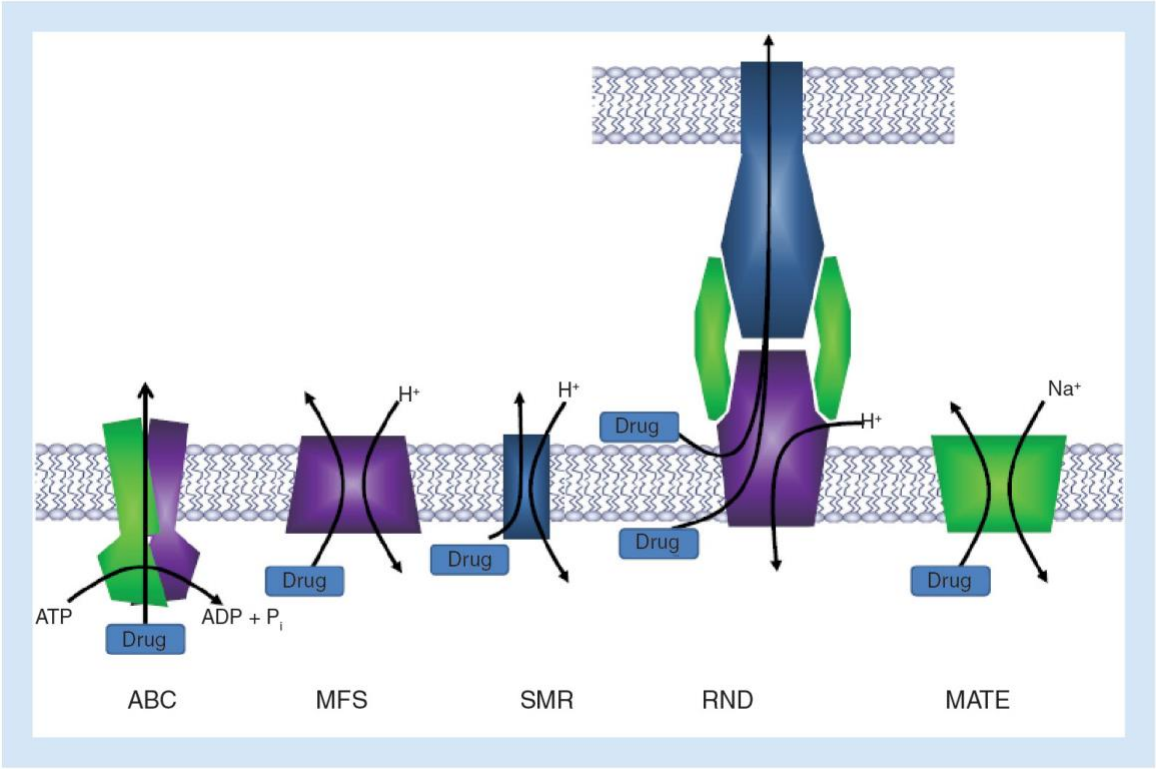

Fig.3: Multi efflux pumps families in Gram-negative bacteria. Efflux pump proteins are able to transport different molecules including antibiotics and harm particles, out of the bacterial cell. Cited from (Blair *et al*., 2014).

Multidrug efflux pumps in *C. jejuni*

Efflux pumps are transporter proteins that are located in the plasma membrane of bacterial cell (Amaral *et al*., 2014). Basically, efflux pumps in bacteria are found as five groups depending on composition, source of energy and number of transmembrane spanning regions as; the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the ATP binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family and the multidrug and toxic compound (Poole, 2007; Piddock, 2006). Biologically, the resistance to antibacterial agent means that agent is unable to affect the target place with the enough concentration that inhibit the activity of the pathogen. The resistance to tetracycline by *E. coli* was the first efflux pump that identified in bacteria (McMurry *et al*., 1980; Zavascki *et al*., 2007). Lately, the sixth bacterial efflux pump family has discovered in bacteria which called the Proteobacterial Antimicrobial Compound Efflux (PACE) superfamily (Hassan *et al*., 2015).

Ability of *C. jejuni* to attack the host cells is belonging to its pathogenicity (Dasti *et al*., 2010). To start infection in people, *C. jejuni* attacks the intestine epithelial layer and colonizes the digestive tract by utilizing an assortment of destructiveness components (Young *et al*., 2007). The CmeABC multidrug resistance (MDR) efflux pump plays a key part in *C. jejuni* colonization of chickens by intervening resistance to bile salts display within the intestinal tract. The MDR pump could be a tripartite efflux framework having a place to the resistance-nodulation-division (RND) superfamily of bacterial transporters. It comprises of three components, i.e., the external layer channel-forming protein CmeC, the internal layer sedate transporter CmeB, and the periplasmic protein CmeA, which bridges CmeB and CmeC (Iovine NM, 2013.). The CmeABC complex contributes to the inherent resistance of *C. jejuni* to a wide run of anti-microbials, heavy metals, and other antimicrobial specialists (Lin *et al*., 2002.).

There are many levels of variety within the amino corrosive groupings of the CmeB protein among *C. jejuni* strains, which may have an effect on the function of this transporter (Cagliero *et al*., 2006). Examination of the atomic components of anti-microbial resistance is critical for control of the spread of multidrug-resistant microbes (Bolton, 2015). Although most considers of MDR pumps have cantered on examinations of their part as anti-microbial resistance determinants, MDR pumps can play a specific role in bacterial pathogenesis (Fernando and Kumar, 2013).

In addition, the CmeB homologues AcrB (Salmonella enterica and Klebsiella pneumoniae) and MexB (Pseudomonas aeruginosa) are required for intrusion of have cells and destructiveness (Webber *et al*., 2009). The CmeABC efflux pump of *C. jejuni* is known to be required for resistance to anti-microbials, bile salts, and a few disinfectants (Mavri and Mozina, 2012).

It can be concluded that the extensive use of antibiotics in medicine has led to an increased incidence of antibiotic resistance in Campylobacter spp. Also, role of multidrug resistance (MDR), the *Campylobacter* CmeABC resistance-nodulation-division (RND)-type efflux pump may be involved in increase its virulence and make the control of this pathogen as a big challenge (Vieira *et al*., 2017). Moreover, the CmeABC efflux pump is involved in acquired resistance of *C. jejuni* to macrolides and tetracycline (Gibreel *et al*., 2007).

Antibiotic resistance in *Campylobacter* spp.

Many Campylobacter strains have the resistance to tetracycline linked to a gene borne by the pTet plasmid (Iovine, 2013). Also, erythromycin resistance is chromosomally mediated in *C. jejuni* and *C. coli* and is caused by ribosome alteration; the resistance mechanism is not consistent with the presence of a rRNA methylase or modification of the ribosome (Taylor *et al*., 1992). In addition, resistance to clarithromycin is caused by a shift in one of two adenine residues in the 23S rRNA of *Helicobacter pylori*, a closely related bacterium. While, fluoroquinolone resistance is caused by a mutation in DNA gyrase A's quinolone resistance deciding area (GyrA) (Taylor *et al*., 1997). The 23S rRNA genes of erythromycin-resistant Campylobacter spp. were sequenced, and mutations at these same locations were discovered, which are most likely responsible for antibiotic resistance (Trieber *et al*., 1999).

In addition, the over-use of antibiotics in the human population and in animal husbandry has led to an increase in antibiotic-resistant pathogens, especially with fluoroquinolones. This is because *C. jejuni* gastroenteritis is hard to recognise clinically from that caused by other pathogens, and these infections are generally treated with fluoroquinolones. Since *C. jejuni* is naturally transformable, acquisition of additional genes imparting antibiotic resistance is likely. Thus, understand of the antibiotic resistance mechanisms in *C. jejuni* is necessary to provide the suitable therapy for veterinary and human populations (Iovine, 2013).

Aminoglycosides are protein union inhibitors of numerous Gram-positive and Gram-negative pathogens. They contain amino-modified sugars, are emphatically charged, water-soluble and have atomic weights extending from 445 to 600. Commonly, utilized individuals of this gather incorporate gentamicin, kanamycin, amikacin, neomycin, tobramycin and streptomycin (Bérdy *et al*., 2006). The introductory official of aminoglycosides to contrarily charged bacterial films is electrostatic in nature and generally moderate compared with the

moment stage of fast but reversible official to the 30S fragment of the ribosome (Taber *et al*., 1987).

Transfer of aminoglycosides across the bacterial cytoplasmic membranes requires oxygen, an intact electron transport system and ATP (Bryan and Kwan, 1983). There are two major implies by which aminoglycosides apply antimicrobial action (1) impedances with the translocation of the early peptide chain from the ribosomal A location to the P location driving to untimely end. (2) impedances with proof-reading, driving to joining of inaccurate amino acids and broken protein. The most instrument of aminoglycoside resistance in *C. jejuni* is by means of aminoglycoside adjusting chemicals, which are as a rule plasmid-borne. Aminoglycoside resistance was to begin with recognized in *C. coli* and was intervened by a 3′-aminoglycoside phosphotransferase that had been represent as conferring kanamycin resistance in Streptococcus and Staphylococcus (Lambert *et al*., 1985).

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