Control of Campylobacter in poultry

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Abstract: The Gram-negative bacterium Campylobacter is the most common bacterial cause of human gastroenteritis. Poultry, particularly chickens, is considered a major source of human campylobacteriosis. Thus, on-farm control of *Campylobacter* in poultry would reduce the risk of human exposure to this pathogen and have a significant impact on food safety and public health. To date, three general strategies have been proposed to control *Campylobacter* in poultry at the farm level: (1) reduction of environmental exposure (biosecurity measures), (2) an increase in poultry's host resistance to reduce *Campylobacter* carriage in the gut (e.g., competitive exclusion, vaccination), and (3) the use of antimicrobial alternatives to reduce and even eliminate *Campylobacter* from colonized chickens (e.g., bacteriophage therapy and bacteriocin treatment). This review is focused on two promising strategies—vaccination and bacteriocin treatment. In particular, we extensively review recent research aimed at discovering and characterizing potent anti-*Campylobacter* bacteriocins to reduce *Campylobacter* load at the primary production level in poultry.

Introduction

Microaerophilic *Campylobacter* spp., including *C. jejuni* and *C. coli*, are the most common bacterial causes of human gastroenteritis in the United States and many industrialized countries **(Tauxe, 2002).** Human *Campylobacter* illnesses are caused primarily by *C. jejuni* ($\square$90%) and secondarily by *C. coli* ($\square$10%). The estimated cases of campylobacteriosis in the United States are more than 2 millions per year **(Mead *et al.*, 1999).** The medical and productivity costs resulting from *C. jejuni* infection are estimated at 1.5–8.0 billion dollars each year in the United States **(Buzby and Roberts, 1997).** Poultry comprises the greatest concentration of *Campylobacter* and thus the main source of human campylobacteriosis (**Friedman *et al.*, 2000)**. A recent study using a novel population genetics approach further indicated that chicken is the major source of *C. jejuni* that is pathogenic to humans, whereas wild animal and environmental sources are responsible for only 3% of campylobacteriosis **(Wilson *et al.*, 2008).** Quantitative risk assessment models have indicated that a reduction of *C. jejuni* numbers on a broiler carcass by 100-fold (or 2 log units) could result in a significant reduction (30 times less) in the incidence of campylobacteriosis **(Rosenquist *et al.*, 2003)**. Therefore, reduction or elimination of *Campylobacter* in the poultry reservoir is an essential step to control this food safety problem. Although there are multiple levels at which *Campylobacter* contamination can be targeted and implemented, on-farm control of *Campylobacter* would have the greatest impact because the intestine of living poultry is the only amplification point for *Campylobacter* throughout the food chain **(Wagenaar *et al.*, 2006 & 2008).**

Campylobacter is highly prevalent in poultry production systems, such as broilers, layers, turkeys, and ducks **(Sahin *et al.*, 2002).**

Hazard identification

Campylobacter is a leading cause of zoonotic enteric infections in most developed and developing countries **(WHO, 2001)**. The reported incidence of *Campylobacter* infections has markedly increased in many developed countries within the last 20-year period. Under-reporting of *Campylobacter* infections is an issue in many countries and incidence rates only reflect the number of laboratory-confirmed cases. The true rate of infection is considered to be higher than the number of reported cases (from 7.6 to 100 times higher) **(Samuel *et al.*, 2004).** Cases are usually caused by *Campylobacter jejuni* and to a lesser extent by *Campylobacter coli* **(Anon., 1999).**

Growth and survival

In general, *Campylobacter* spp. grow at 37°C, but not below 30°C (Table 2.1). It is there fore reasonable to assume that *Campylobacter* spp. do not multiply during processing, post-processing, refrigerated transport and in refrigerated storage. However, the organisms can survive these steps, especially when the temperature is low. On chilled, raw chicken and pork skin, *C. jejuni* and *C. coli* have

been found to survive for several weeks (**Solow,** *et al*.,, **2003).**

Reservoirs

The principal reservoir of pathogenic *Campylobacter* spp. is the alimentary tract of wild and domesticated mammals and birds. Several countries have monitoring programmes to determine the prevalence of *Campylobacter* in food producing animals and birds. The results of these programmes have earlier been reported to WHO and published by the Community Reference Laboratory on the Epidemiology of Zoonoses (BgVV, Berlin) and are currently being reported to and published by the European Food Safety Authority (EFSA). From these reports, it is evident that *Campylobacter* is commonly found in broilers, broiler breeder flocks, cattle, pigs, sheep, wild animals, birds and dogs (Anon. 2001a; 2006b). Other investigations have shown that healthy puppies and kittens **(Hald and Madsen, 1997),** rodents **(Berndtson, 1996),** beetles **(Jacobs-Reitsma** *et al.,* **1995),** and flies **(Nichols, 2005**) may also carry *Campylobacter*. *C. jejuni* is predominantly associated with poultry but has also been isolated from cattle, sheep, goats, dogs and cats (**Anon., 2006b**). *C. coli* is predominantly found in pigs **(Jensen** *et al.***, 2006),** but has also been isolated from poultry, cattle, and sheep **(Anon., 2006b).** A seasonality of broiler flock colonization has been observed in some countries, leading to a peak in flock prevalence during the warm summer months **(Christensen** *et al., ***2001).**

Sources of *Campylobacter* infection of poultry flocks are still debatable. Vertical transmissionvia contaminated eggs has been reported, but strong supporting evidence is lacking. Isolation from eggs has been demonstrated as a rare event. In particular, **Shanker,** *et al.* **(1986)** obtained two positive eggs from a sample of 187 eggs from a *Campylobacter*-positive breeder flock. The occurrence of only two positive samples is attributed to faecal contamination of the eggshell. *Campylobacter* have poor survival rates in egg albumen **(Jones** *et al.,* **1991).** *Campylobacter* colonization is rarely evident before flocks are two weeks of age, so vertical transmission is not likely to be a major route of flock infection, unless the bacteria are slow to revive, grow and spread among the birds, after being in the harsh environment of the egg (**Van De Giessen** *et al.,* **1992).**

In broiler chickens, *C. jejuni* colonization can persist for the lifetime of the animal (6–7 weeks), consequently leading to carcass contamination at the slaughter facility. Together, *Campylobacter* can rapidly disseminate throughout the flock, and establish persistent and high-level colonization in broilers, which greatly challenges the development of effective farm-based intervention measures to reduce *Campylobacter* in poultry.

On-farm intervention measures to reduce *Campylobacter* in poultry have been comprehensively reviewed recently (**Connerton** *et al.***, 2008).**

Vaccination of Chickens Against Campylobacter
Campylobacter infections and chicken host immunity

Through oral ingestion, *C. jejuni* enters the host intestine and colonizes the distal intestine, primarily the cecum in chicken. Although *Campylobacter* was considered a commensal of the avian host, *C. jejuni* infection triggers both a systemic and mucosal immune response in chickens (**de Zoete** *et al.***, 2007).** *C. jejuni*–specific serum IgG, IgA, and IgM, and mucosal IgA and IgG increased after oral infection with *C. jejuni* (**Widders** *et al.***, 1996).** Specifically, *Campylobacter*-specific serum IgG, IgA, and IgM levels were elevated gradually 2–3 weeks after experimental inoculation, and mucosal IgA rose 3–4 weeks after oral infection. The antibodies are directed against multiple *Campylobacter* antigens, among which flagellin is usually the first antigen to be recognized by all antibody iso types (**Rice** *et al.***, 1997).** The elevated levels of *Campylobacter*-specific antibodies are correlated with reduced colonization level of *Campylobacter*, suggesting a protective role of the antibodies in anti-*Campylobacter* infection in chickens. The *Campylobacter* maternal antibodies could also be vertically transferred from infected layer hens to newly hatched chickens (**Sahin** *et al.***, 2001).** The high-level of *Campylobacter* maternal antibodies in young chickens may partly contribute to the lack of *Campylobacter* infection in young broiler chickens in natural environments during the first 2 weeks of life, which was also supported by laboratory challenge experiments (**Sahin** *et al.***, 2003).** Together, these findings demonstrated the protective nature of *Campylobacter*-specific antibodies and supported the feasibility of development of immunization-based approaches to control Campylobacter infections in poultry.

Interaction between *Campylobacter* and chicken immune system

It is not surprising that *Campylobacter*-specific antibody response is slow and moderate in chickens because *Campylobacter* infection in chicken does not cause a strong inflammatory response or tissue damage in intestine. It is still largely unknown how *Campylobacter* interacts with the chicken immune system to trigger the immune response. Understanding the delicate interactions between *Campylobacter* and the chicken immune systems would greatly facilitate development of immunization-based approaches to control *Campylobacter* infections in poultry. In some studies, *Campylobacter* was also isolated from the

spleen, liver, and blood in young chickens, suggesting that *Campylobacter* may invade intestinal epithelial cells and become systemic (**Van Deun *et al.*, 2008**) further demonstrated that *C. jejuni* could adhere to and invade chicken intestinal epithelial cells *in vitro* and *in vivo*. Notably, the *in vitro* invasiveness of *C. jejuni* was correlated with the magnitude of spleen colonization in *C. jejuni*–inoculated chickens. The *C. jejuni* strains that invaded chicken epithelial cells were not able to proliferate intracellularly, but quickly evaded from the cells. Therefore, **Van Deun *et al.* (2008)** proposed a novel colonization mechanism of *C. jejuni* by escaping rapid clearance through short-term epithelial invasion and evasion, combined with fast replication in the mucus. Interestingly, a recent report showed that *C. jejuni* also colonized the bursa of Fabricius of day-old chicks with 10^4–10^7 CFU/g of content in bursa for up to 28 days (**Bingham-Ramos *et al.*, 2008**). Given that the bursa of Fabricius is an important immune organ in chickens, further examination of the colonization of *C. jejuni* in the bursa may provide novel information on the interaction between *Campylobacter* and the host immune system.

Some *in vitro* studies using chicken cells (e.g., primary chicken embryo intestinal cells, primary chick kidney cells, or chicken macrophage cell HD11) also provided compelling evidence that *Campylobacter* could stimulate the expression of proinflammatory cytokines and chemokines in chickens (**Li *et al.*, 2008**). Recently, **Smith *et al.* (2008)** also reported that a significant induction of proinflammatory chemokin transcript was observed in both day-old and 2-week-old chickens upon infection with *C. jejuni*. These *in vitro* and *in vivo* studies indicated that *C. jejuni* could intimately interact with the chicken immune system to trigger an immune response although no pathological signs are observed for *Campylobacter* infection in chickens.

Vaccine development against *Campylobacter* in chickens

Vaccine development against *Campylobacter* in chickens has been comprehensively reviewed by **de Zoete *et al.* (2007)** recently. There is no vaccine available to date to control *Campylobacter* infections in poultry. A successful chicken vaccine should prevent colonization or cause a strong reduction of *Campylobacter* numbers in chickens (>2 log units) (**de Zoete *et al.*, 2007**).

The following three approaches have been explored for developing effective and safe vaccine against *Campylobacter* in poultry:

1. Live attenuated vaccines. Because infection with wild-type *C. jejuni* strain induced anti-*Campylobacter* antibodies (**Widders *et al.*, 1996**), it is likely that a live attenuated vaccine will have a protective effect. However, experimental colonization with a noncolonizing *C. jejuni* strain did not protect upon homologous challenge (**Ziprin *et al.*, 2002**). In addition, the paucity of information on the pathogenesis of the organism complicates this strategy.

2. Killed whole-cell vaccines. This type of vaccine could induce high protective immunity without the concern regarding potential pathogenesis to human. Vaccination with killed *C. jejuni* whole cells enhanced the immune responses and partly reduced colonization of *C. jejuni* in chickens (<2 log) (**de Zoete *et al.*, 2007**).

3. Subunit vaccine. Successful development of subunit vaccine needs improved knowledge on immunogenic and protective antigens in *C. jejuni*.

Several studies have been focused on immunodominant antigen Fla with variable success (reviewed by **de Zoete *et al.*, (2007)**. However, Fla is modified by glycosylation and undergoes both phase and antigenic variation, which limits the application of Fla-based vaccines. The most encouraging vaccination study was published by a Polish group, in which oral vaccination of chickens with CjaA via a *Salmonella* carrier strain reduced *C. jejuni* colonization by 6 logs (**Wyszynska *et al.*, 2004**). However, this finding is intriguing and needs to be confirmed because of the following two reasons. First, only two treatment groups (untreated chicken vs. vaccine treatment) were used in this study, and there was no *Salmonella* carrier strain control group included (**Wyszynska *et al.*, 2004**). Therefore, it is likely that the protective effect observed in this vaccination trial was mediated by general boost of host immunity due to *Salmonella* infection instead of specific anti-CjaA antibodies. Second, a recent study (**Wyszynska *et al.*, 2008**) indicated that CjaA is an N-glycosylated lipoprotein localized in the inner membrane of *C. jejuni*. Thus, it is difficult for specific CjaA antibodies to pass through outer membrane and gain access to CjaA, consequently conferring protection. Regarding CmeC and CfrA, the two promising vaccine candidates, the protective efficacy of these subunit vaccines needs to be determined in chicken in the future.

Oral delivery systems would be appropriate for *Campylobacter* vaccine in poultry as far as cost and simplicity of administration are concerned (**Wagenaar *et al.*, 2008**). Particularly, successful identification of protective antigens as well as epitope mapping will lead to the development of inexpensive and practical oral vaccines for chickens to prevent *Campylobacter* infections using appropriate delivery systems, such as attenuated *Salmonella*-based vaccines (**Curtiss *et al.*, 1989**) and genetically modified *Lactobacillus* (**Mota *et al.*, 2006**). In conclusion, the short average life span

of broiler chickens (□6 weeks) poses a significant challenge to induce a strong antibody response against *Campylobacter* in chickens. To develop an effective vaccine against *Campylobacter* in poultry, three main challenges have been identified: (1) the identification of cross-protective antigens, (2) the induction of rapid and strong immune response, and (3) the development of novel adjuvants to further stimulate immunity against *Campylobacter* **(de Zoete *et al.*, 2007)**.

Bacteriocins to Reduce *Campylobacter* in Poultry Bacteriocins

Bacteriocins are designated as the antimicrobial peptides (AMPs) produced by bacteria with narrow or broad host ranges **(Cotter *et al.*, 2005)**. Bacteriocins are ribosomally synthesized, produced, and exported by almost every bacterial species examined to date for the apparent purpose of destroying their competitors **(Riley and Wertz, 2002)**. Many bacteriocin-producing bacteria (e.g., lactic acid bacteria) are commensals in intestine **(Sit and Vederas, 2008)**. Therefore, the intestinal bacteriocin–producing bacteria may achieve competitive advantage and function as an innate barrier against pathogens in the gut. Bacteriocins are classified into modified bacteriocins (Class I bacteriocins, such as nisin) and unmodified bacteriocins (Class II bacteriocins, such as the anti–*C. jejuni* bacteriocins described below) (Sit and Vederas, 2008). Despite the existence of a broad diversity in bacteriocin sequences and structures, it has been widely accepted that bacteriocins and other host defense peptides share a common theme in the mechanism of killing action by disruption of membrane integrity **(Yeaman and Yount, 2003)**. Generally, AMPs directly interact with target cells via initial electrostatic and hydrogen bond attraction, and then disrupt the structure or function of the bacterial membrane by permeating lipid bilayers, forming a transmembrane pore, and ultimately leading to cell death. However, transmembrane pore formation is not the only mechanism of bacterial killing by bacteriocins **(Sahl and Bierbaum, 2008)**. For example, nisin, a bacteriocin widely used for food biopreservation, also has other modes of antimicrobial action, such as inhibition of cell-wall biosynthesis, inhibition of lipid bilayer function, inhibition of spore outgrowth, and activation of autolytic enzyme **(Sahl and Bierbaum, 2008)**. Detailed information on bacteriocin evolution, structure–function relationships, and mode of action are available in several excellent reviews **(Peschel and Sahl, 2006)**.

Potential of bacteriocins as new antimicrobials

Bacterial pathogens are increasingly resistant to currently available antibiotics, and new antimicrobials are needed to combat multidrug resistance **(Walsh, 2003)**. Bacteriocins have considerable potential for the design and production of new antimicrobials **(Sit and Vederas, 2008)**. In contrast to traditional antibiotics, bacteriocins are considered natural and nontoxic on eukaryotic cells because they are found commonly in food animal products and thus have been consumed for centuries. In fact, two bacteriocins, nisin and pediocin PA1/AcH, have been widely used in the food industry for food biopreservation, and no toxicity due to these bacteriocins has been demonstrated **(Galvez *et al.*, 2007)**.

From standpoint of antimicrobial development, the emergence of bacteriocin resistance is a concern, either for food preservation or for therapeutic treatment. Because nisin is the only bacteriocin licensed as a food preservative and many potential bacteriocins are still under development, limited information is available directly addressing the development and mechanisms of bacteriocin resistance. Both Gram-positive and Gram-negative bacteria can develop resistance to bacteriocins (e.g., nisin), and the mechanism of bacteriocin resistance appears to be complex and involves various structural and physiological changes in the bacterial cell envelope. Intriguingly, it seems that bacteria have not developed highly effective mechanisms to resist natural AMPs, including bacteriocins **(Sahl and Bierbaum, 2008)**. This feature is very different from many therapeutic antibiotics for which bacteria can develop high-level of resistance. Recently, it has been proposed that bacteriocins may have multiple low-affinity targets and cause pleotropic effects on various bacterial targets. Therefore, it is possible that such low-affinity interactions of bacteriocins with multiple targets are not favorable for the development of bacterial resistance. In contrast, many therapeutic antibiotics act on a single, high-affinity target, which makes it comparatively easy for bacteria to develop resistance, particularly high-level resistance **(Sahl and Bierbaum, 2008)**. Together, bacteriocins have considerable potential to fulfill the need for more effective antimicrobial agents. Unlike the antibiotics that act on a single target, there is less in the way of resistance development for bacteriocin-based antimicrobials.

Anti-*Campylobacter* bacteriocins

In the past 3 years, significant progress has been made toward isolation of chicken commensal bacteria inhibitory to *Campylobacter* and characterization of associated bacteriocins from these bacteria (**Nazef *et al.*, 2008**). Several potent anti-*Campylobacter* bacteriocins have been purified and characterized in bacteria isolated from the chicken intestinal tract, which includes SRCAM 602 from *Paenibacillus polymyxa* **(Svetoch *et al.*, 2005)**, OR-7 from

Lactobacillus salivarius **(Stern *et al.*, 2006)**, and E-760 and E 50–52 from *Enterococcus* spp. **(Svetoch *et al.*, 2008)**.

Lactic acid bacteria such as *Lactobacillus* spp. are widely used probiotic organisms. Many lactic acid bacteria produce bacteriocins with different spectra ranges of inhibition **(Galvez *et al.*, 2007)**.

Colonization of *C. jejuni* in poultry

Colonization of the gastrointestinal (GI) tracts by *C jejuni* is the most significant contributing factor in the contamination of poultry meat **(Grant *et al.*, 1980)**.

The organisms are transferred onto the meat during mechanized processing of the birds **(Genigeorgis et al., 1986)**. Reducing colonization levels and prevalence

in broilers during grow-out is an important part of decreasing *Campylobacter* contamination of poultry meat. However, prevalence on processed carcasses is almost always less than in the intestinal tracts of birds during production. There are fluctuations in levels throughout processing. Prevalence and levels of campylobacters on carcasses decrease after scalding but increase again following picking, probably because of cross contamination from the mechanical picker. However, by the time carcasses exit the chill tank, *Campylobacter* spp. levels and prevalence are lower than when they entered the processing plant **(Berrang and Dickens, 2000)**. Therefore, the ideal way to reduce the incidence of human infection would be to significantly reduce the GI colonization of these organisms in broiler chickens.

The GI colonization by *C. jejuni* in birds is very complex and involves interaction of the host and pathogen, which is influenced by many environmental factors. In a study in France, it was found that a variety of factors at farm level increased the risk of occurrence of *Campylobacter* in broiler flocks. These included high temperature and static air in poultry houses, poor water quality, absence of bootdips, and presence of litter-beetles (**Refrégier-Petton *et al.*, 2001)**. Vertical transmission from breeder hensis also a possibility **(Cox *et al*., 2002)**. *Campylobacter* is ecologically adapted to the avian GI tract and selects the ceca for colonization because the microenvironment is conducive to its survival and multiplication **(Beery *et al.*, 1988)**. The organism colonizes the cecal crypt mucus without attaching to the microvilli. It exhibits chemotactic attraction tol-fucose, a component of mucin, and utilizes mucinas a sole substrate for growth **(Beery *et al*., 1988;).** Therefore, changes in mucincomposition are likely to influence *C. jejuni* colonization in the GI tract.

Control by reduction of colonization

Certain strategies, such as competitive exclusion (CE) have been utilized to take advantage of bacterial antagonism and thus reduce the colonization of pathogenic organisms in the GI tract of birds. Interestingly, certain dietary substrates cause changes in mucincomposition, there by influencing the colonization of mucus-dwelling organisms. **Udayamputhoor *et al.* (2003)** compared the effects of three diet formulations containing different protein sources (animal, plant, and a combination of animal and plant) on the colonization of *Campylobacter jejuni* in the GI tract of broiler chickens. The ceca of birds receiving plant based feed had significantly less colonization than the ceca of birds receiving the other types of feed. A strategy that has been tried in preventing colonization of pathogens in the GI tract of birds is the manipulation of indigenous microflora, and reduction of pathogens by CE**. Nurmi and Rantala (1973)** introduced the concept of CE, and reduced the colonization of *Salmonella* in chicks using intestinal flora of adult chickens. The introduction of flora from an adult bird into a day-old chick speeds the maturation process of the gut microflora and increases the resistance of most chicks to colonization by *Salmonella*. **Bailey (1988)** noted that the CE technique showed a slight reduction to as much as a four-fold reduction in the number of salmonellae, and suggested an integrated approach using CE and other control measures at farm level for colonization control of *Salmonella* in poultry. In the subsequent years, the CE approach led to the experimental use of prebiotics, probiotics, and synbiotics for reduction of colonization of enter pathogens in poultry and farm animals. Prebiotics are oligosaccharides that are not hydrolyzed in the small intestine but modify the composition of microflora in the large intestine. The objective of prebiotics is to promote the growth of specific beneficial bacteria such as *Bifidobacterium* spp. **(Collins and Gibson, 1999)**. Probiotics, according to **Fuller (1989)** consist of live microbial feed supplements which beneficially affect the host ani mal by improving its intestinal microbial balance. The major components of probiotics commonly used in farm animals are *Lactobacillus, Bifidobacterium, Streptococcus, Pediococcus, Enterococcus, Bacillus*, yeasts (*Saccharomyces*), and filamentous fungi such as *Aspergillus* and *Torulopsis* **(Berg, 1998)**. Synbiotics are probiotics and prebiotics used in combination. Examples are *Bifidobacterium* with fructooligosaccharide and *Lactobacillus* with lactitol. These combinations may improve survival of the probioticorganism because its specific substrate is readily available for fermentation **(Collins and Gibson,1999)**. However, the results with respect to the effects of prebiotics and probiotics on broiler performance and nutrient utilization is quite variable

and unpredictable. All these strategies, invariably manipulate the gastrointestinal miroflora so that growth of some beneficial organisms is favored to suppress the colonization by pathogens. But some strategies reported to be helpful in reducing the colonization of *Salmonella* spp. have not been found useful in the case of *Campylobacter* spp. *Salmonella* colonizes the epithelium of the lower intestinal tract, mainly the cecum, whereas *Campylobacter* spp. Are found colonizing crypt mucus without attaching to crypt microvilli. *Campylobacter jejuni* does not adhere to or penetrate epithelial cells **(Meinersmann *et al.*, 1991)**. Hence, strategies that target organisms found in the epithelium, such as receptor antagonism may not be the best in reducing colonization of *Campylobacter*. Mucous and crypt dwelling microorganisms have been used alone or in combination with other intestinal bacteria from chickens to competitively exclude *Campylobacter* colonization in poultry. These include mucusadapted, curved bacteria resembling campylobacters called K-bacteria **(Aho *et al.*, 1992)**, and members of *Enterobacteriaceae*, capable of using mucin as solesubstrate for growth, and producing anti-*C. jejuni* metabolites **(Schoeni and Doyle, 1992)**. Intervention strategies that are successful with *Salmonella* spp. have also been found to be somewhat successful in *C. jejuni* colonization reduction, because of the concentration of campylobacters in cecal crypts.

These included avian specific probiotics containing *Lactobacillus acidophilus*, and *Streptococcus faecium* **(Morishita *et al.*, 1997)**. Compared to convention a lCE, use of mucosal CE microflora has recently been found to reduce *Campylobacter* colonization significantly more **(Stern *et al.*, 2001)**. Recently, **Heres *et al.* (2004)** noted that chickens fed acidified feed were somewhat less susceptible to an infection with.

Campylobacter than were chickens fed conventional feed. A combined use of CE strategy with prebioticson a diet designed exclusively of plant origin may contribute a great deal in reducing the colonization of *C. jejuni* in the GI tract of birds. According to the French antimicrobial surveillance data **(Avrain *et al.*, 2003)**, between 1999 and 2002 there was a change in the *C. jejuni*/*C. coli* ratio in the ceca of standard broiler chicken, with a decrease of *C. jejuni*, and proportionate increase of *C. coli*. It will be important to monitor the *Campylobacter* species ratio in the future to determine whether this situation will remain stable. Production factors such as a ban on animal proteins and fat, and most of the growth promoters are hypothetical explanations for this observed phenomenon, but other yet unsuspected factors may explain the species ratio evolution. Furthermore, it is of utmost importance to monitor in the future the variation of *C. coli* in human campylobacteriosis as suggested by **Tam *et al.* (2003)**.

Antimicrobial resistance Fluoroquinolones, such as ciprofloxacin, and macrolides, such as erythromycin, have been the primary antimicrobials used for the treatment of human Campylobacter infections. Resistance to fluoroquinolones requires only one point mutation in the gyr Agene and resistance has increased rapidly among chicken and human Campylobacter isolates since the early 1990s **(Wieczorek and Osek,2013).** Studies have shown a clear positive association between the use of fluoroquinolones in poultry production and increased resistance among chicken and human Campylobacter isolates (**Wieczorek and Osek,2013),** whereas in countries not permitting the use of fluoroquinolonesin poultry production, such as Australia and the Nordic European countries, few resistant Campylobacter isolates are found from chickens and humans with domestically acquired infections **(Garcia-Migura *et al.*,2014)** The USA banned the use of the fluoroquinoloneenro floxacin in chickens in 2005. Despite this, resistance to ciprofloxacin in C. jejuni from chicken slaughter batches has remained stable at 22% between 2005 and 2013, although at retail level, ciprofloxacin resistance decreased from17% in 2005 to 11% in 2013. Moreover, ciprofloxacin resistance in human C. jejuni isolates in 2013 remained at the same level as in 2005 (22%) (http://www.fda.gov/downloads/AnimalVeterinary/Saf etyHealth/AntimicrobialResistance/NationalAntimicro bialResistanceMonitoringSystem/2015). The reasons for persistence of resistance are not well understood. In the EU member states, in 2013, ciprofloxacin resistance among human Campylobacter isolates ranged from 23% in Denmark to 92% in Spain. Resistance to ciprofloxacin canal so be related to foreign travel, especially to Asia; in 2013 it was shown that 90% of the tested isolates originating from Asia were resistant (. Ciprofloxacin resistance among isolates from broilers at slaughter ranged from 0% in Finland to 90% in Spain (Anonymous.,2015). Tetracycline resistance showed similar trends asciprofloxacin in the EU member states, whereas resistance tomacrolides, currently considered the drugs of choice for treatment of human Campylobacter infections, was low, which is probably because of their limited use in poultry production**. Multidrug resistance has been uncommon in Campylobacter derived from both humans and poultry **(Anonymous.,2015).**

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