Risk assessment and new developing strategies to reduce prevalence of *campylobacter* spp. In broiler chicken meat

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1. Introduction

Over the last two decades, *Campylobacter* has emerged as the most commonly reported cause of bacterial enteritis in humans in the UE and most other developed countries. *Campylobacter* spp. contamination of chicken carcasses is common, and poultry is generally recognised to play a significant role in human *Campylobacter* spp. infection. *Campylobacteriosis* remains the most frequently reported zoonotic disease in humans in the EU with an incidence rate of approximately 50 confirmed cases per 100,000 population in over 17 countries. It is estimated that there are approximately nine million cases of human campylobacteraiosis per year in the EU27 (EFSA, 2010, 2011). *Campylobacter* are ubiquitous bacteria, frequently found in the alimentary tracts of animals, especially birds, and commonly contaminate the environment, including water. *Campylobacteriosis* in humans is caused by emerged thermotolerant *Campylobacter* spp. *Campylobacter jejuni* (C. *jejuni*) has recently overtaken *Salmonella* spp. as the major reported source of food-borne bacterial diseases within the European Union. *C. jejuni* has been found associated with biofilms of other bacterial species. Biofilm formation may play a role in the epidemiology of *C. jejuni* infections (Gunther and Chen, 2009). Although it is generally recognized that there are many sources of *Campylobacter*, *Campylobacteriosis* is predominantly believed to be associated with the consumption of poultry meat, especially fresh broiler meat. Over the past decade, Risk Analysis—a process consisting of risk assessment, risk management and risk communication—has emerged as a structured model for improving food control systems, with the objectives of producing safer food and reducing the numbers of foodborne illnesses. Therefore, control of *Campylobacter* spp. commonly focuses on reducing its occurrence in broiler meat. In recent years, several quantitative risk assessments for *Campylobacter* spp. in broiler meat have been developed to support risk managers in controlling this pathogen. The risk assessments are not only used to assess the human incidence of campylobacteraiosis due to contaminated broiler meat, but more importantly for analyses of the effects of control measures at different stages in the broiler meat production chain. Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens and in the elaboration of standards for food in international trade.

Given the public health and economic problem represented by *Campylobacter* spp., it is important to take measures in order to reduce its prevalence throughout the poultry production chain leading to a reduced incidence of the human illness. Several strategies have been applied to reduce *Campylobacter* spp. counts on chicken meat, including attempts to elimination from the farms by increasing biosecurity and the separation of contaminated flocks, and by improving hygiene during the process of slaughtering. In addition, several experimental approaches like the reduction of colonization by competitive exclusion, antibacterial agents, or phage therapy are being investigated for their efficacy. The combination of prebiotics and probiotics to reduce *Campylobacter* spp. are known as symbiotic, and may have antimicrobial activity. It is generally acknowledged that *Campylobacter* spp. is sensitive to acid conditions. Several strategies developed to reduce *Campylobacter* spp. populations are based on the acidification of the pathogen environment or by acidification of drinking water and feed. Although these measures undoubtedly will help to control shedding of *Campylobacter* by the animals and may reduce the number of positive flocks, vaccination of poultry against *Campylobacter* will probably be most effective and remains a major goal. However, several studies have actually pointed out partial association between the veterinary use of antibiotics and the emergence of resistant strains of *Campylobacter* spp. related to human enteritis. In recent years, there has been increased research interest in the use of nonthermal alternative methods for microbial inactivation, such as high hydrostatic pressure or pulsed electric fields (Sagarzazu et al., 2010). The attraction of these technologies lies in the production of microbiologically safe foods with minimal changes in their sensory and nutritional attributes. Consumers demand high quality, natural, nutritious, fresh appearance and convenient meat products with natural flavour and taste and an extended shelf-life. One area of research is the development of new and improved methods of meat preservation. Due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural and in particular, biopreservation and plant extracts, including their essential oils (EOs) and essences (Djenane et al., 2011a,b). It is well established that these natural compounds have antimicrobial properties against *C. jejuni*. This chapter presents a short
review of recent works on the strategies application to prevent or reduce *Campylobacter* spp. contamination in broiler meat.

2. Chicken meat production and the implications for *Campylobacter*

2.1 Primary production

The public health benefits of controlling *Campylobacter* spp. in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. Workman *et al.* (2008) indicate that *Campylobacter* spp. horizontal transmission is the most significant mode of broiler flock colonization. It is widely assumed that farm workers constitute risk factors for colonization of the flock, and that human traffic is an important vehicle for *Campylobacter* being introduced into the poultry house from the external environment. Water in drinkers can also be contaminated and act as a rapid vector of *Campylobacter* spp. within the flock. Once a bird is colonized with *Campylobacter* spp., it will excrete large numbers of the organism in its faeces. Contact with the faeces of such a bird is one mechanism by which the organisms spread throughout a flock. However, the second consequence of this excretion of organisms is the contamination of the exterior of the birds. This external contamination may occur while the birds are on the farm or during transportation to the slaughter facility. It has been shown that *Campylobacter* strains can survive overnight in slaughterhouses surfaces after a cleaning and disinfection procedure and highlight a new putative source of carcass contamination during processing (Peyrat *et al.*, 2008).

![Table 1. Prevalence of *Campylobacter*-contaminated broiler batches, by country and in the EU.](image)

<table>
<thead>
<tr>
<th>Country</th>
<th>Broiler batches</th>
<th>Prevalence (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>661</td>
<td>32</td>
<td>Jacobs-Reitsma <em>et al.</em>, 1995</td>
</tr>
<tr>
<td>Sweden</td>
<td>287</td>
<td>27</td>
<td>Berndtson <em>et al.</em>, 1996</td>
</tr>
<tr>
<td>Iceland</td>
<td>71</td>
<td>25</td>
<td>Birgisdottir <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Denmark</td>
<td>125</td>
<td>50.4</td>
<td>Dang <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Austria</td>
<td>398</td>
<td>56.8</td>
<td>Ursinitsch <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Denmark</td>
<td>8911</td>
<td>42.5</td>
<td>Wedderkopp <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Japan</td>
<td>212</td>
<td>32.1</td>
<td>Chuma <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>France</td>
<td>75</td>
<td>42.7</td>
<td>Refregier-Petton <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>USA</td>
<td>32</td>
<td>87.5</td>
<td>Stern <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Canada</td>
<td>93</td>
<td>60</td>
<td>Nadeau <em>et al.</em>, 2002</td>
</tr>
<tr>
<td>Belgium</td>
<td>446</td>
<td>86.8%</td>
<td>Ghafir <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>France</td>
<td>422</td>
<td>67.1</td>
<td>EFSA, 2010</td>
</tr>
<tr>
<td>Spain</td>
<td>389</td>
<td>88</td>
<td>EFSA, 2010</td>
</tr>
<tr>
<td>Finland</td>
<td>411</td>
<td>3.9</td>
<td>EFSA, 2010</td>
</tr>
</tbody>
</table>

2.2. The slaughter and processing of chicken

The prevalence of flock positivity is directly related to slaughter age. In Sweden, Berndtson *et al.* (1996) found that where the majority of flocks are harvested at 33-35 days of age; increasing the age of slaughter to 42-44 days increased the flock positivity by about two-fold and to 48-61 days by about four-fold. *Campylobacter* spp. can be expected to contaminate the chicken meat during slaughter and processing of chicken as a result of faecal contamination. The process operations that have been found to cause the greatest changes in the contamination are: scalding, de-feathering, evisceration, washing and chilling (Rosenquist *et al.*, 2006). The stunning process has few microbiological implications, although birds may sometimes inhale contaminated water, which can reach internal tissues (Gregory and Whittington, 1992). Although immersion scalding results in a net reduction in carcass contamination, the highly contaminated state of the water offers ample opportunity for microbial transmission between carcasses. The principal microbiological problem with de-feathering is cross-contamination of carcasses associated with the mechanical action of the machines, and the tendency to disperse microbial contaminants in all directions via aerosols. Evisceration is a critical stage where bacteria can be spread in poultry processing (Perko-Mäkelä *et al.*, 2009). In most cases, the levels and numbers of carcasses contaminated can significantly increase (ICMSF, 1980). Of major importance is the need to minimize rupture of the exposed intestines and prevent the spread of faecal *Campylobacter*, which is present in relatively high numbers in the intestines of positive birds. Washing has been found to produce a ten-fold reduction in *Campylobacter* (Cudjoe *et al.*, 1991), but it would be expected to have little effect on cells attached to carcass surfaces. *C. jejuni* has been found to form and attach onto biofilm in the poultry industry (Nguyen *et al.*, 2011) thus being an important source of contamination of final products. Biofilms may provide the ideal niche for *C. jejuni* survival, as there are
microenvironments in the biofilm that are believed to provide ideal conditions for development. A reduction in the *Campylobacter* spp. concentration on chicken carcasses may also be obtained by interventions aimed at reducing the concentration of *Campylobacter* spp. in the intestines of the living birds (Rosenquist et al., 2006).

### Table 2. Prevalence of *Campylobacter*-contaminated broiler carcasses, by country and in the EU, 2008 (EFSA, Journal, 2010).

<table>
<thead>
<tr>
<th>Country</th>
<th>Broiler batches</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>408</td>
<td>80.6</td>
</tr>
<tr>
<td>Denmark</td>
<td>396</td>
<td>31.4</td>
</tr>
<tr>
<td>Estonia</td>
<td>102</td>
<td>4.9</td>
</tr>
<tr>
<td>Germany</td>
<td>432</td>
<td>60.8</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>Portugal</td>
<td>421</td>
<td>70.2</td>
</tr>
<tr>
<td>Spain</td>
<td>389</td>
<td>92.6</td>
</tr>
<tr>
<td>Sweden</td>
<td>410</td>
<td>14.6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>401</td>
<td>86.3</td>
</tr>
</tbody>
</table>

### 2.3 Distribution, retail, consumer handling and preparation

Many papers have reported on the level of contamination with *Campylobacter* spp. in retail poultry meats and/or by-products. For example, the prevalence of *Campylobacter* spp. was reported to be 32.0–43.0% in Germany (Adam et al., 2006), 50.5–73.5% in the UK (Meldrum et al., 2006), 79.0% in the USA (Nannapaneni et al., 2005), and 62.4% in Canada (Valdivieso-Garcia et al., 2007).

The majority of *Campylobacter* infections are acquired via the oral route after handling raw poultry or consuming undercooked poultry. Seasonality has been found to influence the *Campylobacter* prevalence in retail chicken meat (Boysen et al., 2011). Contaminated chickens are most abundant during summer and early autumn. At home, during meal preparation, individuals can be exposed to *Campylobacter* from fresh chicken through a large number of pathways. These pathways include: direct contamination from the chicken to any food commodities not undergoing a subsequent cooking step before ingestion; indirect contamination of surfaces upon which cooked products or ready-to-eat food are placed; contamination directly onto hands and subsequent ingestion; insufficient cooking; and a wide variety of other potential contamination events. Transfer can be facilitated by liquid carried on hands, utensils and cutting boards. Unsafe food handling procedures in private kitchens are assumed to be responsible for a large number of cases of food-borne diseases in most countries (Zhao et al., 1998).

### 3. *Campylobacter* risk assessments and hazard identification

The pathogenesis of *Campylobacter* has been reviewed (Wooldridge and Ketley, 1997). Motility, chemotaxis and the flagella are known to be important factors in the virulence as they are required for attachment and colonization of the intestinal epithelium (Ketley, 1997). Once colonization has occurred, *Campylobacter* may perturb the normal absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion and/or production of toxin(s), or indirectly, following the initiation of an inflammatory response (Wooldridge and Ketley, 1997). The exposure assessment initially evaluates the frequency and levels of *Campylobacter* on the farm, estimating the probability that a random flock is *Campylobacter*-positive, the within-flock prevalence and the levels of colonization and contamination of the broilers. Subsequently, the stages of transport, processing, storage and preparation by the consumer are explored, and combined to predict the overall impact that these stages will have upon the contaminating *Campylobacter* load on a random chicken carcass or product, and to determine the final exposure level. Hazard characterization describes the adverse health effects of organism. This component of the risk assessment usually includes a dose-response relationship. This is represented as a probability that a random member of the population will become infected or ill after exposure to a specific number of *Campylobacter* organisms. The types of data that can be used to establish dose-response relationships include animal and human feeding studies, and epidemiological data, such as data from outbreak investigations. Populations at risk with respect to infectious diseases often include the elderly, children and individuals suffering from illnesses that compromise their immune systems (e.g. AIDS and cancer patients). As birds are prepared, caught, loaded, transported to processing plants and finally slaughtered, they undergo stresses that may affect not only broiler meat quality, but also its microbiological status (EFSA, 2011). The normal, balanced gut microflora in animals provides reasonable protection against colonization with pathogens. Stress in animals can disturb this status, weaken the immune responsiveness, and cause an increase in shedding of pathogens. Hence, stress management is a relevant aspect of pathogen control. During transportation to the slaughterhouse, and
while waiting to be slaughtered, the stress of crowding birds in close proximity in crates is likely to further disseminate *Campylobacter* contamination present in faeces, and on the skin and feathers or released from the gastrointestinal tract. Spreading untreated farm wastes containing enteric pathogens as fertilizers on pasture or agricultural land for crop production can mediate further infections or re-infections of animals with pathogens through contaminated grazing, harvested feed or water supply (Hutchison *et al*., 2004).

### Table 3. Growth characteristics of thermophilic *Campylobacter* species (ICMSF, 1996).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Optimum</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>40–42°C</td>
<td>&lt; 30°C – &gt; 45°C</td>
</tr>
<tr>
<td>pH</td>
<td>6.5–7.5</td>
<td>&lt; 4.9 – &gt; 9.0</td>
</tr>
<tr>
<td>O₂</td>
<td>3 – 5%</td>
<td>0 – 15 to 19%</td>
</tr>
<tr>
<td>CO₂</td>
<td>10%</td>
<td>-</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.997</td>
<td>&lt; 0.987</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5%</td>
<td>&gt; 2 %</td>
</tr>
</tbody>
</table>

#### 3.1. Antibiotic-resistant

The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter* (Helms *et al*., 2005), which has potentially serious impact on food safety in both veterinary and human health. The antimicrobial resistance, especially to fluoroquinolone, ciprofloxacin, increased in many *Campylobacter* species. This is particularly seen as a risk for fluoroquinolone resistant *Campylobacter* (Geenen *et al*., 2010), and the use of antimicrobials to control *Campylobacter* in broilers is strongly discouraged. Andersen *et al*., (2006) found that raw food samples from the retail level represent an important sampling point, which reflects the consumer exposure to resistant *C. jejuni* originating from raw poultry.

#### 3.2 Disease description

The most common pathogenic species for humans are *C. jejuni* and *C. coli*. Motility by the flagellum is required for efficient colonisation of intestinal cells. Cell invasion is a major pathogenic mechanism of *Campylobacter* infection. Campylobacters have been shown to produce cytotoxic proteins that may play a role in clinical courses of the disease. Apart from causing diarrhoea, Campylobacters can cause neurological complications such as Guillain-Barré syndrome (GBS) and the Miller Fisher Syndrome. Evidence suggests an association between *Campylobacter* illness and a rare but serious paralytic condition, GBS, a demyelinating disorder of the peripheral nervous system resulting in weakness, usually symmetrical, of the limbs, weakness of the respiratory muscles and loss of reflexes. Early symptoms of GBS include burning sensations and numbness that can progress to flaccid paralysis (Yan *et al*., 2005). GBS has been estimated to occur about once in every 1000 cases of campylobacteriosis (Allos, 1997). The syndrome is correlated with prior infection by *C. jejuni* in up to 40% of cases (Nachamkin *et al*., 2000). It has been shown that GBS arises as a result of autoimmune attack due to molecular mimicry that exists between certain lipopolysaccharide (LPS) molecules of *C. jejuni* strains and human nerve tissue gangliosides. Furthermore, some serotypes of *C. jejuni* are associated with GBS. Reactive arthritis (incomplete Reiter Syndrome) has been estimated to occur in approximately 1% of patients with campylobacteriosis. The symptoms occur seven to ten days after onset of diarrhoea (Peterson, 1994).

#### 3.3 Sources of illness and risk factors

A recent study showed that the number of food-borne gastrointestinal infections in humans associated with pathogens has increased worldwide in recent years. Although salmonellosis still are the most common pathogens in most industrialized countries, campylobacterioses are continuously increasing and have already overtaken the salmonelloses in the EU (EFSA, 2010). The probability of illness is dependent on the occurrence of three conditional probabilities: the probability that the organism is ingested; the probability that the organism is able to survive and infect the host once it is ingested; and the probability of the host becoming ill once infected. The environment, the pathogen and the host are all variables that play an important role in the probability of illness. Environmental factors include the food vehicle and the stability of the gastrointestinal tract ecosystem. Pathogen factors include the dose, virulence, and the colonization potential in the host gastrointestinal tract. Host factors include immune status, age and stomach contents (Coleman and Marks, 1998). The primary risk in developed countries is considered to be the food related risk factors. There is no doubt that poultry is a major source of *Campylobacter* spp. and there is scope for cross-contamination of other foods if contaminated poultry is introduced into the kitchen. Chicken meat preparations of minced meat, marinated or seasoned wings or filets… are a new food type introduced in the UE market and gaining growing popularity by consumers. They may contain skin due to their nature or because of sensory characteristics (Uyttendaele *et al*., 2006). All of these items have in common that they have been manipulated extensively during processing and as such also have a potential for...
Campylobacter contamination levels not only on the surface of the meat but also interior to the meat preparation. Harris et al. (1986) observed an association between infection and not washing the kitchen cutting board with soap. Other food-related risk factors that have repeatedly been identified include consumption of other meat types, undercooked or barbecued meat, raw seafood, drinking untreated surface water, or unpasteurized milk or dairy products (El-Sharoud, 2009). Eating meat cooked outside the home has also been identified as a risk factor. Other food items that have been related to sporadic cases of human campylobacteriosis are contaminated shellfish (Harris et al., 1986). Simulation showed that eating raw chicken meat products can give rise to exposures that are $10^{10}$ times higher than when the product is heated, indicating that campaigns are important to inform consumers about the necessity of an appropriate heat treatment of these types of food products (Uyttendaele et al., 2006).

The importance of poultry as a risk factor for human cases has been demonstrated in countries where interventions have been implemented in the broiler production chain or where poultry has been withdrawn from the market, and where a decline in human cases has followed. For example, in Belgium, due to the dioxin crisis in 1999, where all poultry meat and eggs were withdrawn from the market, the estimated reduction of campylobacteriosis cases following this event was 40% within the crisis period (Vellinga and Van Lock, 2002). Different studies have shown associations between sporadic human cases and animal sources, suggesting that these sources would be the most important risk factors for sporadic human infections. Seasonal variation in Campylobacter prevalence in broilers, with a peak in the summer has been previously reported from several countries in northern Europe (Hofshagen and Kruse, 2003). The reason for the association of higher Campylobacter prevalence and season is not known, but appears to be temperature-related. During summer, ventilation and water consumption also increase because of the higher temperatures. Human infections show similar seasonality in many European countries. There is strong evidence for frequent cross contamination during slaughtering and product processing.

4. Usual prevention methods

The poultry industry should be receptive to new interventions that could be applied at different stages during processing. Many poultry processors have increased the use of chlorine and water and have incorporated intervention strategies to reduce Escherichia coli and Salmonella spp. on carcasses to comply with the pathogen reduction and HACCP regulations. However, these strategies may have little impact on the reduction of Campylobacter populations. Strict implementation of biosecurity in primary production and Good Manufactured Practices/Hazard Analysis Critical Control Points (HACCP) during slaughter may reduce colonization of broilers with Campylobacter.

Prevention of horizontal transmission on-farm. This control measure is generally known by the term “biosecurity” and is concerned with protecting birds from infection from outside sources. This is a very important control measure; because once Campylobacter enter a poultry flock the spread can be rapid and currently impossible to control. Practical biosecurity measures at the farm level have been determined as the primary strategy to prevent colonisation of housed broiler flocks with Campylobacter entering the processing plant and hence the food chain. Campylobacter enters the broiler house from the exterior environment, and the most important control measure is to prevent or, more realistically, to limit this entry (Hermans et al., 2011). The limited action of hygiene procedures is based on the fact that in conditions where broilers are confronted with environmental factors that are scarcely controllable, biosecurity is difficult to apply. In these production systems, Rivoal et al. (2005) have shown that, even if strict hygiene measures allow broiler flocks to be Campylobacter-negative during the first weeks of age, birds are almost always colonized at slaughter, after the access of birds to the open-air range. Another factor linked to biosecurity is the quality of the drinking water. Several studies have found that drinking water of poor quality is related to an increased risk of a flock being positive for Campylobacter (Sparks, 2009).
**Vaccination.** Vaccination of animals, particularly when combined with other measures implemented further along the food chain, can be an efficient strategy for pathogen reduction. Assuming that biosecurity can never be fully effective, the vaccination of poultry to reduce susceptibility to Campylobacter could be used to support biosecurity measures (De Zoete et al., 2007). Vaccination might reduce or even prevent colonization and in both cases would affect the numbers of organisms entering the food chain and the environment. It is also important to ensure that the vaccine delivery is cost effective (Humphrey et al., 2007).

**Antibiotic use.** The use of antibiotics in modern intensive animal production as growth-promoters and for therapy and prevention of diseases could not be a rational solution to reduce Campylobacter incidence. Several studies have actually pointed out the partial association between the veterinary use of antibiotics and the emergence of resistant strains of Campylobacter related to human enteritis (Luangtongkum et al., 2006).

**Competitive exclusion.** These products consist of bacterial collections from the microbiota of adult chickens and are given to young chicks to prevent colonization by Campylobacter species. Competitive exclusion concept can be applied, primarily in monogastric animals. This involves feeding with complex mixtures of bacteria that reduce attachment of pathogens to the gut mucosa. Competitive exclusion flora is a concept taking advantage of bacterial antagonism to reduce animal intestinal colonization by pathogenic microorganisms (Schneitz, 2005). Commensal gut flora may be manipulated by changing the diet of the animal and some research has shown that chickens given certain diets are more prone to resist challenge with campylobacters.

**Bacteriophage therapy.** Bacteriophage therapy is one possible means by which Campylobacter colonization might be controlled, thus limiting the entry of Campylobacter into the human food chain (Carrillo et al., 2011). Similarly, experiments suggest that treating live birds with specific bacteriophages shortly prior to slaughter may be an effective control measure (Havelaar et al., 2007). Recently, there has been a renewed interest in the use of bacteriophages as ‘therapeutic’ agents; a prerequisite for their use in such therapies is a thorough understanding of their genetic complement, genome stability and their ecology to avoid the dissemination or mobilisation of phage or bacterial virulence and toxin genes (Timms et al., 2010).

**Bacteriocins.** Other method to reduce the Campylobacter spp. load in poultry is the use of bacteriocins as a therapeutic treatment for chickens colonized by Campylobacter (Svetoch et al., 2003). By feeding the animals therapeutic feed at the appropriate moment in the cycle, levels and frequency of colonization can be reduced, which may be effective in lowering the human health risk imposed by Campylobacter. Lin (2009) has reviewed anti-Campylobacter bacteriocins for potential use in reducing the numbers of Campylobacter (jejuni as well as coli) in poultry. Stern et al. (2006) found that control chickens (standard feed) were colonized in the caecum with 6.6–8.3 \( \log_{10} \) CFU of Campylobacter per g, while all treated chickens (feed modified with purified bacteriocin) contained undetectable numbers (< 2 \( \log_{10} \) CFU/g). Svetoch et al. (2005) administered bacteriocin E 50–52 to young chicks. High levels of C. jejuni were found in the control chicks (8.40 \( \log_{10} \) CFU/g of caecal contents), while no Campylobacter was detected in the treated group. Thus, it seems that bacteriocins, administered just before slaughter, can reduce Campylobacter colonization in the chicken caecum to undetectable levels.

**Probiotic.** To decrease the risk of human infection, Campylobacter should be controlled at farm levels. Orally given probiotic bacteria could prevent colonisation of chicken with pathogenic Campylobacter (Morishita et al., 1997). Chaveerach et al. (2004) found that Lactobacillus (P93) strain isolated from conventional chicken had potential inhibitory activities against all tested Campylobacter. Probiotics can be incorporated in the diet. This is based on feeding with viable microorganisms antagonistic toward pathogens via either modifying environmental factors in the gut or producing antimicrobial compounds (Morishita et al., 1997).

**Prebiotics and synbiotics.** Combinations of prebiotics and probiotics are known as synbiotics, and may have antimicrobial activity (Klewricki and Klewricka, 2004). Foeks and Gibson (2002) have yet recorded a C. jejuni inhibition in vitro, with a population reduction below detectable level after 24 h culture, with a Lactobacillus plantarum or Bifidobacterium bifidum, when combined with oligofructose or an oligosaccharide.

**Litter acidification.** Acidification of poultry litter has also been suggested as a method to limit pathogen proliferation in breeding flocks (Line, 2002). Campylobacter was not detected in unused litter (Jacobs-Reitsma et al., 1995) probably because the lack of moisture renders it a very hostile environment for the organism. Thus fresh litter is an unlikely source of contamination in the house.

**Freezing.** Freezing of chicken products during post-processing has been shown to reduce the level of carcass contamination and is used as part of the control strategy in some countries (Sampers et al., 2010). Rapid freezing of
Irradiation. Chun et al. (2010) investigated the applicability of UV-C irradiation (wavelengths of 220–300 nm) on the inactivation of C. jejuni in ready-to-eat meat and poultry meat respectively. The results have clearly indicate that UV-C irradiation effectively decreased C. jejuni inoculated on meat during storage. Irradiation of food materials, using electron beams (from electron accelerators) or high-energy electromagnetic radiation (gamma-rays from 60Co or X-rays), is permitted in some European countries and will inactivate campylobacters and other infectious bacteria (Humphrey et al., 2007). The application of irradiation in poultry at doses of 1-10 kGy eliminates pathogenic bacteria (Lacroix and Ouattara, 2000). However, irradiation might have some effects on the organoleptical quality of meat products. The threshold dose above which off-flavors are detected in irradiated meats was reported to be 2.5 kGy for poultry (Henis et al., 1989). Natural antioxidants from spices could be employed to stabilize fats and control oxidative deterioration of foods during irradiation. The effect of the combination of irradiation and marinating with rosemary and thyme extract on the sensitivity of pathogen and sensory characteristics of poultry has also been investigated. A dose of 2-3 kGy would be sufficient to decontaminate meat from campylobacters (Ingram and Farkas, 1977; Monk et al., 1995). However, application of this technology has been very limited. A disadvantage in the European Union is that at present the use of gamma-irradiation for meat is strongly discouraged. Its limited use appears to be due to distrust by the public of any process which depends on the nuclear industry as well as lack of knowledge by the public in general concerning food borne infections and the effectiveness of irradiation. A preferred option might be to use electron accelerators which require no isotope. These are used, particularly in UE, to decontaminate raw chicken portions (Carry et al., 1995). Kampelmacher, (1984) showed that a dose as low as 1 kGy was effective in reducing C. jejuni by more than 4 log-cycles with this dose. The directive 1999/3/EC contains a list of foodstuffs authorized for irradiation treatment and the doses allowed. So far, only dried aromatic herbs, spices and vegetable seasonings are included in the list. However, irradiation of other foodstuffs including poultry is temporarily permitted in some Member States. In the United States, FDA and USDA have approved irradiation of poultry meat at a maximum dose of 3 kGy to control foodborne pathogens such as Campylobacter (Keener et al., 2004).

Chemical treatments. It is generally acknowledged that Campylobacter is sensitive to acid conditions. Several strategies developed to reduce Campylobacter populations are based on the acidification of the pathogen environment. Application of organic acids via drinking water has produced various results. Byrd et al. (2001) found that the prevalence of Campylobacter spp. in broiler crops was reduced from 85% to 62% by application of lactic acid in drinking water during the ten-hour feed withdrawal. Animal health and welfare were not affected by the acid application. A pH of around 4.0 proved to be most effective against Campylobacter when formic, acetic, propionic, and hydrochloric acids were tested in vitro as additives to a mixture of water and feed, alone or in combination (Chaveerach et al., 2002). Skanseng et al. (2010) found that a combination of 1.5% formic acid and 0.1% sorbate reduced the colonization of C. jejuni significantly, while a concentration of 2.0% formic acid in combination with 0.1% sorbate prevented C. jejuni colonization in chickens. Acidifying bacteria, particularly lactic acid bacteria (LAB) may also contribute to preserve food. Nevertheless, their antimicrobial properties are not limited to the food industry field. Several in vitro and in vivo studies have investigated the bacterial antagonistic activities against Campylobacter (Chaveerach et al., 2004; Svetoch et al., 2005). The authors have suggested that the inhibitory effect of Lactobacillus (P93) on Campylobacter growth could be explained mainly by organic acids production, resulting in pH reduction. Then, several studies have pointed out the bactericidal activity of hydrogen peroxide (H2O2) produced by LAB in the presence of oxygen (Strus et al., 2006). Besides organic acids and H2O2, bacteriocins are the third kind of compounds that may help to inhibit Campylobacter growth, as shown by Stern et al. (2006) for a bacteriocin produced by a Lactobacillus salivarius strain. The antimicrobial activity of organic acids is based on the ability of their undissociated form to penetrate through the cell membrane and to dissociate inside the cell, decreasing the intracellular pH value, thus disrupting homeostasis which is essential for the control of ATP synthesis, RNA and protein synthesis, DNA replication and cell growth (Hirshfield et al., 2003). Riedel et al. (2009) used formic and lactic acids to inactivate C. jejuni inoculated on chicken skin. Riedel et al. (2009) dipped inoculated pieces of skin into 10% trisodium phosphate for 15 sec and found that numbers were reduced by about 1 log10 cycle compared to dipping in water. Arritt et al. (2002) found that the commercial use of an effective antimicrobial chemical spray may help to reduce the level of Campylobacter on raw poultry carcasses and reduce the volume of rinse water applied for carcass washing. Chlorine dioxide (ClO2) is a powerful oxidizing and sanitizing agent, with a broad biocidal activity against bacteria, yeasts, viruses, fungi and protozoa (Gomez-Lopez et al., 2009). When 100 ppm ClO2 was used on chicken carcasses for 10 min, C. jejuni was reduced by 0.9–1.2 log cycles (Hong et al., 2007). Doyle and Waldroup (1996) found that ClO2 added to chill water in two commercial broiler processing plants reduced Campylobacter spp. concentrations by 90%. The effectiveness of electrolyzed water for killing C. jejuni on poultry was also evaluated. Complete inactivation of C. jejuni occurred within 10 s after exposure to electrolyzed water or chlorinated water, both of which contained 50 mg/l of residual chlorine.
Results demonstrated that electrolyzed water was very effective not only in reducing the populations of *C. jejuni* on chicken, but also could prevent cross-contamination of processing environments (Park et al., 2002). Hot water treatment of broiler carcasses may result in reductions in *Campylobacter* counts varying from 0.27 and 1.5 log₁₀ units (EFSA, 2011). Most of these chemicals have been investigated in laboratory studies by inoculating samples of skin, meat or whole carcasses, and then dipping them into solutions of the chemicals (Riedel et al., 2009). Immersion is a very effective method of ensuring full coverage of a product (Hong et al., 2007). In some countries, chlorine, as hypochlorite, has traditionally been used at levels of 50 ppm and higher in the water used during poultry processing, including in the water for immersion chilling (EFSA, 2011). Automatic spraying is the alternative method of applying chemicals to chicken carcasses.

5. New developing strategies against *Campylobacter* in food

**Essential oils.** Increased consumer demand for all natural food products has put pressure on industry and regulatory agencies to closely examine the potential for use of natural antimicrobials that prevent or control the growth of foodborne pathogens and spoilage microorganisms. Although many studies have indicated that EOs have the potential to be used as a natural antimicrobial preservative in the food industry (Djenane et al., 2011a,b), the success in simple agar diffusion systems has not been seen in foods because the antimicrobial activity of EO is reduced in the presence of fat and protein (Burt, 2004). It is generally supposed that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of the EO in some way (Tassou et al., 1995). In one of such studies, an increase in concentration of 10-fold when used in pork sausages, 50-fold when used in soup and 25 to 100-fold when used in soft cheese, 2-fold when used in minced beef and chicken was required to produce a similar effect to that reported in vitro (Djenane et al., 2011c,d). Also the oils may have been less effective on the chicken skin because of the rough surface of the skin, which allowed greater adhesion by the bacteria (Fisher and Philips, 2006). EOs, as antimicrobial agents present two main characteristics: first is their natural origin, which means more safety for consumers, and second is that they are considered to be of low risk for resistance development by pathogenic microorganisms.

![Fig.1](image-url) Inhibition growth of *C. jejuni* by *L. graveolens*, *L. nobilis*, *P. lentiscus* and *S. montana* EOs at 2 fold MICs values in chicken meat stored under microaerobic conditions: (_listing) control; (●) *S. montana*; (▲) *P. lentiscus*; (■) *L. graveolens*; (●) *L. nobilis*. The error bars represent standard deviation (Djenane et al., 2011d).

Coriander EO was tested *in vitro* for antimicrobial activities against *Campylobacter jejuni* using disk diffusion and minimal inhibitory concentration determination assays. It was found that the oil at concentration of 0.5% v/w killed all of the bacteria on the meat, while 0.1% and 0.25% v/w oils reduced the bacterial cell loads on the meat from 5 log cfu/ml to 3 and 1 log CFU/ml respectively (Rattanachaikunsopon and Phumkhachorn, 2010). Antimicrobial activities of the EOs of various herbs were investigated by Abdollah et al. (2010) against *C. jejuni* and *C. coli* isolated from chicken meat. The results indicated that the EOs of these plants exerted remarkable activity against *C. jejuni* and *C. coli* and, therefore, they could be used as natural anti-*Campylobacter* additives in meat. Several recent studies described in detail the antimicrobial properties of some EOs against *C. jejuni*, which may be envisaged as natural alternatives to chemical-based antibacterial for food safety and preservation (Nannapaneni et al., 2009; Djenane et al., 2011d). Despite the potential of many common plants and EOs is considerable, knowledge of this area and studies on their biological
activities remain scarce. Most of the data published on the antimicrobial properties of plant EOs are fragmented and employ only basic screening techniques. Moreover, most studies on the antimicrobial action of plant extracts have been conducted *in vitro*, so that little information exists regarding the antimicrobial activity of EOs in food systems (Burt, 2004). Tested EOs showed promising antibacterial activity against target bacteria. Djenane *et al.* (2011d) support the possible use of *Inula graveolens*, *Laurus nobilis*, *Pistacia lentiscus* and *Satureja montana* EOs, particularly that from *I. graveolens*, for the preservation of chicken meat. By using this method, chicken meat can be stored in a modified atmosphere assuring a low risk associated to *Campylobacter*, at the same time that lipid oxidation is inhibited, giving rise to a higher sensory quality. The ability of *I. graveolens* to inhibit *C. jejuni*, which are gram-negative bacteria, makes it more interesting for use to prevent food-related illness caused by *C. jejuni* and other gram-negative bacteria. Aslim and Yucel (2008) found that the EO obtained from *Origanum minutiflorum* showed strong antimicrobial activity against all of the tested ciprofloxacin-resistance *Campylobacter* spp. The same suggest that the essential oil of *O. minutiflorum* may be used as a natural preservative in food against food-born campylobacteriosis. Many studies have demonstrated that higher concentrations of EOs are required in food systems than in *in vitro* investigations (Djenane *et al.*, 2011a). The use of EO vapours may be a potential way of combating the organoleptic effect brought about by direct contact between the food and EO. However, longer exposure to the vapour is required to produce a similar inhibitory effect (18 h as against 60 s) which has cost implications for the food industry (Fisher and Philips, 2006).

**Wine.** Isohanni *et al.* (2010) suggest that wines could be used as antimicrobial ingredients together with the addition of further antimicrobial agents in meat marinades to reduce the numbers of *Campylobacter* in naturally contaminated poultry products, thus lowering the risk of *Campylobacter* cross-contamination and transmission through food. According to Gañan *et al.* (2009), wine constitutes an adverse environment for the survival of *C. jejuni*. Furthermore, it would be interesting to study the possible use of phenolic compounds in wine as an alternative to the use of antimicrobial growth promoters against these bacteria in broilers.

**Nonthermal methods.** In recent years, there has been increased research interest in the use of nonthermal alternative methods for microbial inactivation, such as, high hydrostatic pressure or pulsed electric fields. The attraction of these technologies lies in the production of microbiologically safe foods with minimal changes in their sensory and nutritional attributes. Sagarzazu *et al.* (2010) shown that incubation of heat-treated cells in the presence of sodium pyruvate highly improved the survival ability of *C. jejuni*; on the contrary, it did not enhance survival ability of this microorganism after exposure to pulsed electric fields treatments.

**Active packaging.** Interest in the use of active packaging systems for meat and meat products has increased in recent years (Kerry *et al.*, 2006). Changes in consumer preferences have led to innovations and developments in new packaging technologies. Active packaging is useful for extending the shelf life of fresh, cooked and other meat products. Forms of active packaging relevant to muscle foods include; O$_2$ scavengers, CO$_2$ scavengers and emitters, drip absorbent sheets, antioxidant and antimicrobial packaging (Camo *et al.*, 2011). Recently, Sánchez-González *et al.* (2011) found that antimicrobial films were prepared by incorporating different concentrations of various EOs, into chitosan and hydroxypropylmethylcellulose films. Their antibacterial effectiveness against pathogens bacteria was studied at 10 °C during a storage period of 12 days. Hydroxypropylmethylcellulose-EO and chitosan-EO composite films present a significant antimicrobial activity against the pathogens considered.

**Combined methods.** Study of Smigic *et al.* (2010) highlighted the importance of combining decontamination technologies with subsequent storage under O$_2$-rich atmosphere, at low pH and low temperature to the control survival and growth of *C. jejuni*. The combination of heat and acid pH was one of the first combined processes used by the food industry, with the objective of reducing the intensity of heat treatments. This practise has the advantage of decreasing heat resistance of *C. jejuni*, but also of preventing the growth of survivors (Palop *et al.*, 1999). Gálvez *et al.* (2010) found that Application of natural antimicrobial substances (such as bacteriocins) combined with novel technologies provides new opportunities for the control of pathogenic bacteria, improving food safety and quality. Bacteriocin-activated films and/or in combination with food processing technologies (high-hydrostatic pressure, high-pressure homogenization, in-package pasteurization, food irradiation, pulsed electric fields, or pulsed light) may increase microbial inactivation and avoid food cross-contamination. Piskernik *et al.* (2011) found the synergistic effect of freezing and rosemary extract antimicrobial activity. As the combination of pre-freezing and plant extract treatment reduced the *C. jejuni* cell number by more than 2.0 log reduction.

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