

Review

A review and future potential approach for *Campylobacter* control in retail poultry meats

Djamal Djenane^{1*}, Javier Yangüela² and Pedro Roncalés²

¹Université Mouloud MAMMERI. Département des Sciences Alimentaires. Faculté des Sciences Biologiques et des Sciences Agronomiques. Laboratoire de Qualité & Sécurité des Aliments. BP 17, 15000 Tizi-Ouzou, Algeria.

²Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/Miguel Servet, 177-50013 Zaragoza, Spain.

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Campylobacteriosis is considered the most frequent zoonosis in humans, and the handling and/or consumption of poultry meat are considered the main source for human infection. Moreover, largely owing to the recent food authority ban on the use of antibiotic growth promoters in animal feed, it is now very important to look for new effective strategies to reduce the incidence of these bacteria in the host. Chicken intestines, and also the intestines of other animals, are the only sites where *Campylobacter* proliferates in meat. Therefore, the development of a novel approach for controlling *Campylobacter* could be a very valuable alternative strategy in the fight to eliminate these bacteria from the poultry meat chain.

Key words: Poultry, meat, retail, *Campylobacter*, control, novel approach.

INTRODUCTION

Campylobacter contamination of poultry carcasses is common, and chicken are generally recognised to play a significant role in human *Campylobacter* infection (Raut et al., 2012; Torralbo et al., 2014). Campylobacteriosis remains the most frequently reported zoonotic disease in humans in the European Union (Table 1). It is estimated that there are approximately nine million cases of human campylobacteriosis per year in the EU 27 (EFSA, 2010, 2011; Kvalsvig et al., 2014).

Some studies show that more than 98% of products derived from raw chicken in shops could be contaminated with this bacterium (Jacobs-Reitsma et al., 2008).

Campylobacter are ubiquitous bacteria, frequently found in the alimentary tracts of animals, especially birds and commonly contaminate the environment, including water (Figure 1).

Campylobacteriosis in humans is caused by emerged thermotolerant *Campylobacter* spp. these pathogens are a leading cause of zoonotic enteric infections in most developed and developing nations worldwide. *Campylobacter jejuni* has recently overtaken *Salmonella* spp. as the major reported source of food-borne bacterial diseases within the European Union (Table 2).

A number of countries have instituted successful

*Corresponding author. E-mail: djenane6@yahoo.es, d.djenane@hotmail.com. Tel: 00 (+213) 779 001 384. Fax: 00 (+213) 26 21 68 19.

Table 1. Reported campylobacteriosis confirmed in humans (EFSA, 2010, 2011).

Country	Cases/100,000 inhabitants
Austria	51.4
Belgium	47.9
Bulgaria	0.2
Cyprus	2.9
Czech Republic	193.3
Denmark	63.4
Estonia	11.5
Finland	84.0
France	5.4
Germany	78.9
Hungary	54.7
Ireland	39.8
Italy	0.4
Latvia	0.0
Lithuania	22.5
Luxembourg	90.7
Malta	18.8
Poland	0.7
Romania	0.1
Slovenia	44.2
Spain	11.4
Sweden	83.8
The Netherlands	39.2
The United Kingdom	90.9

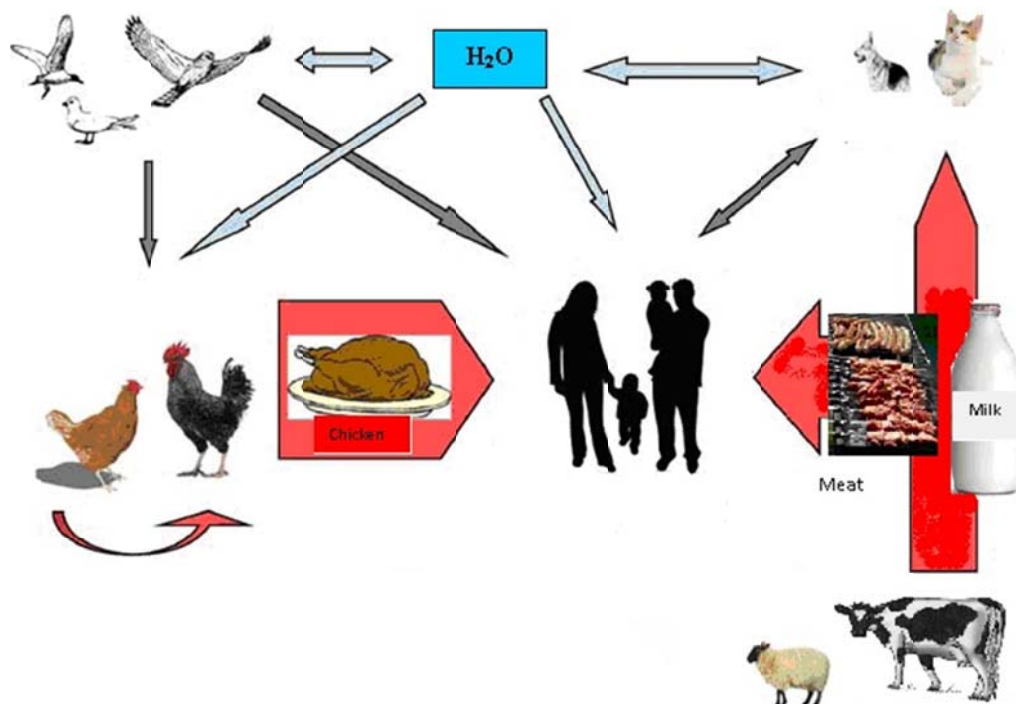


Figure 1. *Campylobacter* spp. sources and risk factors for human illness.

Table 2. Prevalence of *Campylobacter*-contaminated broiler carcasses in the EU (EFSA, 2010).

Country	Prevalence (%)
Austria	47.8
Belgium	31.0
Bulgaria	29.6
Cyprus	30.6
Czech Republic	61.3
Denmark	19.0
Estonia	2.0
Finland	3.9
France	76.1
Germany	48.9
Hungary	50.1
Iceland	25.0
Ireland	83.1
Italy	63.3
Latvia	41.0
Lithuania	41.5
Luxembourg	100
Malta	96.8
Norway	3.2
Poland	78.9
Portugal	82.0
Romania	77.0
Slovenia	78.2
Spain	88.0
Sweden	13.2
Switzerland	59.0
The Netherlands	24.4
The United Kingdom	75.3

prevention and surveillance measures against *Campylobacter* infections. However, campylobacteriosis is challenging to study and some aspects remain poorly understood (Kvalsvig et al., 2014; Macritchie et al., 2014). *C. jejuni* has been found to be 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* spp., campylobacteriosis is predominantly believed to be associated with the consumption of poultry meat, especially fresh broiler meat (Table 3).

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 food-borne illnesses (Miliotis et al., 2014). Therefore, control of *Campylobacter* spp. commonly focuses on reducing the occurrence of *Campylobacter* in broiler meat. In recent

years, several quantitative risk assessments for *Campylobacter* in broiler meat have been developed to support risk managers in controlling this pathogen (Comin et al., 2014). The risk assessments are not only used to assess the human incidence of campylobacteriosis 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 food-borne pathogens and in the elaboration of standards for food in international trade. Given the public health and economic problem represented by *Campylobacter*, it is important to take measures in order to reduce *Campylobacter* prevalence throughout the poultry production chain leading to a reduced incidence of the human illness. Several strategies have been applied to reduce *Campylobacter* counts on chicken meat, including attempts to eliminate *Campylobacter* from the farms by increasing biosecurity and the separation of contaminated flocks, and by improving hygiene during the process of slaughtering (Sasaki et al., 2014). In addition, several experimental approaches like the reduction of colonization by competitive exclusion, antibacterial agents, or phage therapy are being investigated for their efficacy (Timms et al., 2010). The combination of prebiotics and probiotics to reduce *Campylobacter* are known as symbiotic, and may have antimicrobial activity (Klewicki and Klewicka, 2004). 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 or by acidification of drinking water and feed (Chaveerach et al., 2002). 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 the 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* related to human enteritis (Luangtongkum et al., 2006).

In recent years, there has been increased research interest in the use of no thermal 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. Several relatively recent studies describe in detail the antimicrobial properties of wine against *C. jejuni*. The results indicate that the exposure of contaminated food to wine, as in marinade conditions, significantly reduces the number of viable cells of *C. jejuni* (Isohanni et al., 2010). Consumers demand high quality, natural, nutritious, fresh appearance and convenient meat products with natural flavour and taste

Table 3. Risk factors associated with enteric *Campylobacter*.

Risk factors	References
Drinking untreated water, drinking raw milk, eating undercooked chicken, cat in household.	Hopkins et al. (1984)
Eating undercooked chicken, eating pre-packed sandwiches, consumption of raw milk, consumption of mushrooms.	Harris et al. (1986)
Eating undercooked chicken, daily contact with cat, consumption of raw milk. Eating undercooked chicken.	Deming et al. (1987; Schmid et al. (1987) Kapperud et al. (1992)
Eating undercooked chicken, use of untreated water. Travel abroad, eating undercooked chicken.	Ikram et al. (1994) Schorr et al. (1994)
Handling raw meat, contact with animals with diarrhoea, consumption of untreated water.	Adak et al. (1995)
Eating undercooked chicken and consumption of chicken prepared at restaurant, travel abroad, Drinking unpasteurized milk, contact with animals.	Eberhart-Phillips et al. (1997)
Foreign travel, eating chicken, drinking milk from bottles damaged by birds, consumption of medication Omeprazole.	Neal and Slack (1997)
Drinking unpasteurized milk, consumption of chicken, consumption of pork with bones, barbecuing, daily contact with chickens, living or working on a farm.	Studahl and Andersson (2000)
Eating chicken prepared commercially Travel abroad, eating chicken prepared commercially	Effler et al. (2001) Rodrigues et al. (2001)
Consumption of undercooked poultry, consumption of red meat at a barbecue, Consumption of grapes. Drinking unpasteurized milk. Travelling abroad.	Neimann et al. (2003)
Consumption of untreated water, consumption of undercooked poultry. Contact with poultry.	Kapperud et al. (2003)
Contact with poultry Travel abroad, Consumption of chicken prepared at restaurant (and other meats). Food handling and consumption of undercooked problematic chickens. Eating roasted chicken meat and Russian salad. Consumption of salad.	Potter et al. (2003) Friedman et al. (2004) Osiriphun et al. (2012) Calciati et al. (2012); Signorini et al. (2013)

Table 4. Growth characteristics of thermophilic *Campylobacter* species (Park, 2002).

	Optimum	Inhibition
Temperature	40–42°C	< 30°C – > 45°C
pH	6.5–7.5	< 4.9 – > 9.0
O ₂	3–5%	0 – 15 to 19%
CO ₂	10%	–
N ₂	85%	–
Water activity	0.997	< 0.987
NaCl	0.5%	> 2 %

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, bio preservation and plant extracts, including their essential oils (EOs) and essences. It is well estab-

lished that these natural compounds have antimicrobial properties against the human enteropathogen *C. jejuni*.

This paper presents the short review of recent works on the strategies application to prevent or reduce *Campylobacter* contamination in poultry meat.

CAMPYLOBACTER

Campylobacter cells are Gram-negative spirally curved rods. In general, *Campylobacter* species do not grow in conventional aerobic or anaerobic culture systems. *Campylobacter* are O₂-sensitive micro-aerophilic bacteria (Table 4), with optimal growth in an atmosphere containing 5-10% O₂ and 1-10% CO₂, which is related to its niche in the avian tract (Park, 2002). They do not ferment or oxidize sugars and are sensitive to hydrogen peroxide and superoxide anions produced in media. *C. jejuni* and *C. coli* are distinguished from most other *Campylobacter* species by their high optimum growth temperature (42°C). The *Campylobacter* genus has 17 species, 14 of

Table 5. Prevalence (%) and species of *Campylobacter* in retail meat under modified atmosphere packaging (MAP) or unpackaged (Lynch et al., 2011).

Meat	Packaging	No. of samples	Positive (%)
Beef	MAP	92	36 (39)
	Unpackaged	94	30 (32)
subtotal		186	66 (36)
Pork	MAP	91	21 (23)
	Unpackaged	88	19 (22)
subtotal		179	40 (22)
Chicken	MAP	55	9 (16)
	Unpackaged	130	21 (16)
subtotal		185	30 (16)

which have been associated with human illnesses, and of these, *C. jejuni* and *C. coli* cause more than 95% of the infections attributed to this genus (Park, 2002).

This combination of strict requirements places *C. jejuni* in the unique group of food-borne pathogens which are not able to multiply outside of the host and grow in food during either processing or storage. The bacterial cells react to temperature downshift by altering cell morphology and physiology. As the temperature decreases, coccoid cells are formed, resulting in viable but non-cultivable forms. This is considered to be an adaptive response to hostile external environments (ICMSF, 1996). The resuscitation of non-cultivable cells has been demonstrated in chickens (Stern et al., 1994). Even though *C. jejuni* does not grow below 30°C, the bacterium survives on raw meat surfaces at refrigerated temperatures and thus poses a risk to the consumer (Ligowska et al., 2011). Superoxide dismutase plays an active role in the protection against oxidative stress and aerotolerance and is an important factor for survival of *Campylobacter* in food (Park, 2002). *Campylobacter* are particularly sensitive to drying and reduced pH. In addition, *Campylobacter* is sensitive to salt concentrations above 1.5%. *C. jejuni* and *C. coli* are sensitive to heat and do not survive cooking or pasteurization temperatures with D-values of 0.21–2.25 min at 55–60°C (ICMSF, 1996).

ANTIBIOTIC-RESISTANCE

The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter*, which has potentially serious impact on food safety in both veterinary and human health (Messad et al., 2014; Abay et al., 2014). The antimicrobial resistance increased, especially to a fluoroquinolone, ciprofloxacin, in many *Campylobacter* species (Cakmak and Erol, 2012; Lazou et al., 2014).

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.

RETAIL POULTRY MEATS

Many papers have reported on the level of contamination with *Campylobacter* spp. in retail poultry meats and/or by-products (Table 5). 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), 62.4% in Canada (Valdivieso-Garcia et al., 2007) and 62.9% in southern Spain (Torrallbo et al., 2014).

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; Cakmak and Erol, 2012). *Campylobacter* contamination in chicken is highest during summer and early autumn. In the home, during meal preparation, individuals can be exposed to *Campylobacter* from fresh chicken through a large number of pathways. These pathways could 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 and these mechanisms may be a significant contributor to exposure and food-borne illness. 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). Lynch et al. (2011) demonstrate that retail meats contain a much more diverse range of *Campylobacter*, particularly on beef and pork products. The incidence of *Campylobacter* on beef (36%) was significantly higher than on pork (22%) or chicken (16%), and far exceeds previously reported prevalence levels.

It has been found that polyphosphates present in exudates processed chicken, were determined to be largely responsible for the improved survival of *Campylobacter* spp. Therefore, polyphosphates used to enhance chicken quality aid in sustaining the numbers of *Campylobacter* bacteria, increasing the opportunity for disease via cross-contamination or improperly cooked poultry (Nereus and Gunther, 2010). Organic and other

no conventional broiler products are now readily available for retail in many countries, yet very little is known about the status of these broiler flocks with regard to the prevalence of *Campylobacter*.

NEW DEVELOPING STRATEGIES AGAINST CAMPYLOBACTER

Primary production

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 better able to resist challenge with campylobacters.

Bacteriophage therapy is one possible means by which the colonization could be controlled, thus limiting the entry of campylobacters 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). 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).

Other method to reduce the *Campylobacter* load in poultry is the use of bacteriocins from bacteria as a therapeutic treatment for chickens colonized by *Campylobacter*. Svetoch and Stern (2010) reviewed bacteriocin application to reduce the cecal *Campylobacter* counts in broiler chickens of colonized flocks. 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 log₁₀ cfu/g of *Campylobacter*, while all treated chickens (feed modified with purified bacteriocin) contained undetectable numbers (< 2 log₁₀ cfu/g). Svetoch et al. (2008) administered bacteriocin to young chicks. High levels of *C. jejuni* were found in the control chicks (8.40 log₁₀ 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.

Supplementing bacteriocin in drinking water at 3.5-25 mg per bird for three days before slaughter was most effective, resulting in a complete elimination of *C. jejuni* in 90% of the cases. The safety of these bacteriocins was confirmed by conducting experiments on monkey and human cell cultures as well as in treated mice and chickens.

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). Santini et al. (2010) reported both marked *in vitro* and *in vivo* activity for *Bifidobacterium longum* towards *Campylobacter*. Recently, Wang et al. (2014) suggested that *Lactobacillus* strains N8, N9, ZL4 and ZL5 could be used as potential probiotics in food applications against *C. jejuni* infection.

With the ban of dietary antimicrobial agents, the use of probiotics, prebiotics and synbiotics has attracted a great deal of attention in order to improve intestinal health and control food-borne pathogens, which is an important concern for the production of safe meat and meat products. Combinations of prebiotics and probiotics are known as synbiotics, and may have antimicrobial activity (Klewicki and Klewicka, 2004). Fooks 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. Finally, addition of mannanoligosaccharide to the feed of naturally infected birds and xylanase to the feed of artificially infected broilers, as prebiotics, resulted both in a minor, although significant decrease in cecal *C. jejuni* counts in these animals (Baurhoo et al., 2009). The study of Baffoni et al. (2012) highlighted the positive effect of the synbiotic approach for *C. jejuni* reduction in broiler chickens, which is of fundamental importance for the safety of poultry meat consumers. The galactooligosaccharide was then combined with a probiotic *Bifidobacterium* strain (*Bifidobacterium longum* subsp. *longum* PCB133), possessing antimicrobial activity against *C. jejuni*.

In chicken meat

Reducing human *Campylobacter* infection cases has become a priority for the UE Governments. However, the public's views on acceptability of interventions to reduce *Campylobacter* in poultry production are poorly understood

in the UE and in other countries around the world. Overall, findings indicate that increasing consumer acceptability of the most effective interventions is likely to be a difficult process (Macritchie et al., 2014).

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) showed 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.

Irradiation

Haughton et al. (2012) found that exposure of skinless chicken fillet to near ultraviolet/visible light (NUV-vis light: 395±5 nm) for 1 or 5 min at 3 cm distance reduced *C. jejuni* by 2.21 and 2.62 log₁₀ cfu/g, respectively. 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 indicated 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 kill campylobacters and other infectious bacteria (Sparks, 2009). The application of irradiation in poultry at doses of 1-10 kGy eliminates pathogenic bacteria (Lacroix and Ouattara, 2000). Raut et al. (2012) found that radiation treatment with a dose of 1 kGy could achieve complete elimination of 10⁵ cfu of *Campylobacter*/g in poultry meat samples. However, irradiation might have some effects on organoleptic 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 (Hanis 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 organoleptic 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 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).

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 has the potential to be used as a natural antimicrobial preservative in meats (Djenane et al., 2011a, b; 2012a, 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 ways (Tassou et al., 1995). In one of such study, 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., 2011a, 2012b; Tassou and Nychas, 1996). Also, the oils may have been less effective on the chicken skin because of the rough surface of the skin, which allowed for greater adhesion by the bacteria (Fisher and Phillips, 2006). EOs, as antimicrobial agents, present two main characteristics: the first is their natural origin which means more safety for consumers and the second is that they are considered to be low risk for resistance development by pathogenic microorganisms. Kurekci et al. (2013) found that EOs and related terpenoid compounds can have strong anti-*Campylobacter* activity without adversely affecting the fermentation potential of the chicken-caeca microbiota. EOs and their active compounds may have the potential

Table 6. Effect of different phenolic compounds present in wine on the viability of *C. jejuni* using concentration of 1000 mg/L for n = 4 (+ indicates a significant difference with respect to control) (Gaňan et al., 2009).

Phenolic compound	Concentration (mg/L) = 1000
Caffeic acid	+
Catechin	-
Cumaric acid	+
Epicatechin	+
Ferulic acid	+
Gallic acid	+
Methyl gallate	+
p-Hydroxybenzoic acid	+
Quercetin	-
Synaptic acid	+
Tryptophol	+
Vanillic acid	+

to control *C. jejuni* colonisation and abundance in poultry.

In vitro studies have demonstrated the efficacy of different natural substances such as the EOs of cedar wood, jasmine, marigold, ginger, patchouli, carrot seeds, celery, spikenard (Friedman et al., 2002) and orange (Nannapaneni et al., 2009) as antimicrobial compounds with activity against some strains of *C. jejuni*. However, they have not yet been demonstrated to effectively control this pathogen in chickens. Coriander EO was tested *in vitro* for antimicrobial activities against *C. jejuni* using disk diffusion and minimal inhibitory concentration determination assays, it has been noted that coriander oil exhibited the strongest antimicrobial activity against tested *C. jejuni*. In evaluating the antimicrobial potency of coriander oil against *C. jejuni* on chicken meat, it was found that the oil at concentration of 0.5% v/w killed all the bacteria on the meat, while 0.1 and 0.25% v/w oils reduced the bacterial cell loads on the meat from 5 to 3 and 1 log cfu/mL, respectively (Rattanachakunsopon and Phumkhachorn, 2010).

Antimicrobial activities of the EOs of various herbs were investigated by Abdollah et al. (2010) against *C. jejuni* and *Campylobacter coli* isolated from chicken meat. The results indicated that the EO of these plants displayed 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 (Bakkali et al., 2008; Solomakos et al., 2008; Djenane et al., 2011a, 2012a). 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. By using disc diffusion assay, Wannissorn et al. (2005) and Djenane et al. (2012b) evaluated the antimicrobial activity of various EO samples extracted from various plants against *C. jejuni*. Tested EOs showed promising antibacterial activity against target bacteria. Djenane et al. (2012b) 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 the described method, chicken meat can be stored in a modified atmosphere assuring a low risk associated with *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 other Gram-negative bacteria. Aslim and Yucel (2008) found that the EO obtained from *Origanum minutiflorum* showed strong antimicrobial activity against all the tested ciprofloxacin-resistant *Campylobacter* spp. It also suggests that the EO of *O. minutiflorum* may be used as a natural preservative in food against food-borne disease, such as Campylobacteriosis. Many studies have demonstrated that higher concentrations of EOs are required in food systems than *in vitro* investigations (Djenane et al., 2011a, 2012b). 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 Phillips, 2006).

Grape seed extract and wine

Silván et al. (2013) investigated the effects of grape seed extract on the inactivation of *C. jejuni*, the results have clearly indicated that the antibacterial activity against *C. jejuni* of the collected fractions showed that phenolic acids, catechins and proanthocyanidins were mainly responsible for the behaviour observed. Isohanni et al. (2010) suggested 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 Gaňan et al. (2009), wine constitutes an adverse environment for the survival of *C. jejuni* (Table 6). 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.

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 oxygen scavengers, carbon dioxide scavengers and emitters, drip absorbent sheets, antioxidant and antimicrobial packaging (Camo et al., 2011). 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 presented 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 O₂-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 practice 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. The combination of pre-freezing and plant extract treatment reduced the *C. jejuni* cell number by more than 2.0 log reduction.

CONCLUSION

Campylobacteriosis is considered the most frequent zoonosis, and the handling and/or consumption of chicken meat is considered the main source for human infection. The reduction of the rates of infection in chickens should

make an effective contribution to substantially controlling the illness in humans. However, the increase of the general concern about the spreading of antibiotic resistance in humans has determined the elimination of antibiotics as growth promoters in livestock. At this point, it is essential to search for new, natural and sustainable strategies to reduce the incidence of this bacterium in poultry meat. Since chicken intestines, and also the intestines of other animals, are the only sites where *Campylobacter* proliferates in the food chain, it is essential to control the pathogen at these locations. The solution to the problem of *Campylobacter*-contaminated chicken by developing strategies must be economically viable, sustainable and legal, as well as acceptable to the consumer.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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