Prevalence and risk factors for thermotolerant species of *Campylobacter* in poultry meat at retail in Europe

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ABSTRACT The thermotolerant species *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis* are the causative agents of the human illness called campylobacteriosis. This infection represents a threat for the health of consumers in Europe. It is well known that poultry meat is an important food vehicle of *Campylobacter* infection. As emerged from the reported scientific literature published between 2006 and 2016, poultry meat sold at retail level in Europe represents an important source of the pathogen. The contamination level of poultry meat sold at retail can vary depending on pre- and post-harvest factors. Among the pre-harvest measures, strict biosecurity practices must be guaranteed; moreover, among post-harvest control measures scalding, chilling and removal of faecal residues can reduce the contamination level of *Campylobacter*. An additional issue is represented by increasing proportion of *Campylobacter* isolates resistant to tetracyclines, ciprofloxacin, and nalidixic acid, thus feeding a serious concern on the effectiveness of antibiotic treatment for human campylobacteriosis in a near future.

Key words: campylobacteriosis, retail, antibiotic resistance, chicken rearing, meat safety

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CAMPYLOBACTER AND CAMPYLOBACTERIOSIS

The species today ascribed to the genus *Campylobacter*, once classified as *Vibrio* spp., consist of Gram-negative spirally curved rods with a polar flagellum located at one or both ends of the cell (Jay et al., 2005). All the species belonging to the genus are oxidase and catalase positive, non-spore forming and unable to grow in the presence of 3.5% NaCl. Due to its microaerophily, *Campylobacter* requires small amounts of oxygen (3–6%) for growth, whereas its multiplication is inhibited at 21% oxygen. The growth temperature of *Campylobacter* cells is comprised between 37°C and 45°C, with an optimum at 42°C (Forsythe, 2010). Indeed, most species are typical thermotrophics, while they are generally recognised as sensitive to heat treatments (pasteurization and proper cooking). However, as reported by Park (2002), it has been found that cells exposed to temperature values above the optimal for growth may respond through the synthesis of heat shock proteins. This latter phenomenon is a well known homeostatic mechanism leading to bacterial thermotolerance, which is referred to bacteria surviving at 60+/−0.1°C for one hour (Singh, 2009).

The thermotolerant species *Campylobacter jejuni* (the most frequent), *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*, are the causative agents of the human illness called campylobacteriosis, although non-thermotolerant species such as *Campylobacter fetus* may occasionally cause the infection (Forsythe, 2010). The symptoms of the disease generally include diarrhoea, fever, and abdominal cramping, septic arthritis can be sometimes observed, although with low frequency (Altekruse et al., 1999). Acute neuromuscular paralysis, due to the demyelating disorder called Guillain-Barré syndrome, and reactive arthropathy called Reiter syndrome may represent rare complications of campylobacteriosis (Allos, 1997). Except for the above cited cases, the infection is usually self limiting and rehydration, together with electrolytes replacement, are the therapies most commonly prescribed (Allos, 1997); notwithstanding, in some patients (i.e. with high fever, and/or with deficiencies in the immune response) the use of antibiotics as macrolides (e.g. erythromycin), and fluoroquinolones (e.g. ciprofloxacin) is recommended (WHO, 2011; Wieczorek and Osek, 2013). On the other hand, as reported by European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), a high proportion of *Campylobacter* isolates from humans are resistant to ciprofloxacin and tetracyclines, as well as to macrolides, aminoglycosides, and betalactams (Wieczorek and Osek, 2013; EFSA and ECDC, 2015a). This of course leads to decreasing options for

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treatment and control of infections and also arises further concern linked to the possibility that the genes responsible for these resistances are harboured by mobile (transferable) genetic elements and can therefore be widespread among (pathogenic) bacteria (Clementi and Aquilanti, 2011). Accordingly, Crespo et al. (2016) have recently discovered a novel plasmid conferring kanamycin (*aph*A-3) and tetracyclin (*tet*O) resistance in a *C. jejuni* strain. This finding suggests the possibility of a horizontal transfer of the resistance genes between different *C. jejuni* and *C. coli* strains and ecosystems. Given the severe implications linked to the risk of horizontal diffusion of antibiotic resistance genes, the presence of these determinants has been considered in many studies concerning *Campylobacter* isolated from poultry meat.

THE EUROPEAN REGULATORY FRAMEWORK

Diseases caused by foodborne pathogens constitute a major burden to consumers, food business operators and governments (Codex Alimentarius Commission, 1997). In particular, human zoonoses represent a priority problem in Europe since 1992 when the Council Directive 92/117/EEC issued specific monitoring and protection measures concerning these diseases. Subsequently, in 1998, the Decision 2119/98/EC provided for the establishment of the Community network for the surveillance of transmissible diseases in humans, included zoonoses. Finally, Directive 2003/99/EC, that repealed and replaced Council Directive 92/117/EEC, reiterated the need of a careful monitoring of zoonoses and zoonotic agents, and underlined the deficiency of proper epidemiological investigation concerning foodborne outbreaks. A list of the zoonoses and zoonotic agents to be monitored was published in Annex I of the Directive, including: tuberculosis in cattle, brucellosis in cattle, sheep and goats, and certain zoonotic agents in food (in particular *Salmonella, Campylobacter*, and verotoxinogenic *Escherichia coli*). The year after (2004), the European Parliament and the Council issued the so-called "Hygiene Package" that reaffirmed the relevance of risk assessment on the side of the food business operator, while appointing the public authorities with new responsibilities. Thereafter, Regulation (EC) No 2073/2005 (as amended by the Regulation (EC) No 1441/2007) provided microbiological criteria for some food-borne bacteria, microbial toxins and metabolites in food products and processes. Moreover, in order to improve the efficiency of monitoring of the major biological risks in Europe, the Commission created a network of National Reference Laboratories (NRLs) co-ordinated by Community Reference Laboratories (CRLs), responsible for setting up European Union (EU) wide standards for testing, routine procedures and reliable testing methods, thanks to the presence of scientific and technical expertise of excellence especially focussed within the areas of animal health, public health and zoonoses. Originally, Annex VII of Regulation (EC) No 882/2004 listed twelve CRLs for feed and food, responsible for the following subjects: milk and milk products; zoonoses (*Salmonella*); marine biotoxins; viral and bacteriological contamination of bivalve molluscs; residues (four laboratories); transmissible spongiform encephalopathies (TSEs); additives for use in animal nutrition; Genetically Modified Organisms; material intended to come into contact with foodstuffs. Today, the list of CRLs, now called European Reference Laboratories (EURLs), includes laboratories working in the area of animal welfare and other policy areas, such as cosmetics, industrial chemicals and pharmaceuticals. As well as *Campylobacter* is concerned, the National Veterinary Institute (SVA) in Uppsala was the appointed EURL for *Campylobacter* by the European Commission, DG Health and Consumer Protection, on the 1st of July 2006. Its activities include: i) organization of proficiency tests to be carried out at the NRLs to detect and quantify *Campylobacter* spp. in different types of matrices, as faeces, food and environmental samples; ii) organization of annual workshops to inform the NRLs about ongoing activities that include *Campylobacter* at EU and national levels; iii) evaluation and development of analytical methods for the detection, identification and characterization of *Campylobacter* as well as other issues that are important for increasing the knowledge about *Campylobacter* in the food chain; iv) providing scientific and technical assistance to the European Commission and to the NRLs; v) communication and cooperation with public health laboratories.

Currently, European data on zoonoses, both in the veterinary and human fields, are published in the annual reports, edited by the EFSA, which provide the general framework of the European situation.

The collection of data is essential to identify which animals and foodstuffs are the main sources of infection and to monitor the prevalence of zoonoses. The data analysis is also pivotal to support the actions undertaken to prevent and reduce the impact of zoonoses in the food chain. To this end, all EU Member States (MSs) collect and analyze information about foodborne zoonoses in order to protect human health. As reported by the EFSA Panel on Biological Hazards the estimated cost in the EU of the foodborne disease caused by thermotolerant species of *Campylobacter* is around € 2.4 billion a year, in terms of loss of productivity and public health expense (EFSA Panel on Biological Hazards, 2011).

OCCURRENCE OF *CAMPYLOBACTER* IN POULTRY MEAT AT RETAIL

Poultry meat still represents the most important source of food-borne *Campylobacter* (EFSA and ECDC, 2015b). Indeed, in accordance with Article 5 of the already cited Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, a baseline survey to estimate the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses at slaughterhouse level was carried out in the EU (EFSA, 2011). Following a request from the European Commission, the EFSA Panel on Biological Hazards was asked to deliver a scientific opinion on *Campylobacter* in broiler meat production. This opinion was published in 2011 and included control options and performance objectives and/or targets at different stages of the food chain. Regarding the costs of campylobacteriosis, the above cited opinion stated that broiler meat may account for 20 to 30% of the total, while 50 to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens) (EFSA Panel on Biological Hazards, 2011). In the last report jointly published by EFSA and ECDC (EFSA and ECDC, 2015b), 30.7% of fresh broiler meat subjected to microbiological analyses in the year 2014 were positive for the presence of *Campylobacter*, with important differences between the involved EU MSs. As well as the presence of *Campylobacter* in animals is concerned, the above cited EFSA and ECDC report stated that the microorganism was found in 31.8% of slaughter batches and 30.3% of flocks. The EFSA and ECDC (2015b), highlighted 29 strong-evidence food-borne outbreaks among the 422 *Campylobacter* outbreaks recorded; the catering services were recognised as the most relevant source of these outbreaks, as also reported by Osimani and Clementi (2016), but handling at home was also proved to play an important role.

The food retail sector is the point of contact between producers and consumers, where food retailers may have a non marginal role, after that played by breeders and food business operators, to ensure the supply of safe food. Indeed the risk of microbial pathogens contamination at retail greatly varies depending on the scale and the scope of the business: on the one hand, at the large retailers, the risk of contamination is probably lower than at small scale butcheries, however its possible effects can reach a much higher number of consumers (De Boeck et al., 2016).

As the meat sector is concerned, poultry meat sold at retail represents one of the most important vehicles for the diffusion of *Campylobacter* infection (Bardoň et al., 2011; Lynch et al., 2011; Petruzzelli et al., 2014). This infection still represents a threat for the health consumers in Europe, given that in 2014 the number of reported confirmed cases of human campylobacteriosis in EU was 236,851 with an increase of 22,067 cases compared with 2013. Notwithstanding, epidemiological studies on the prevalence of *Campylobacter* in poultry meat are still limited (Andrzejewska et al., 2015).

The present survey ideally follows the review published by Suzuki and Yamamoto (2009) on *Campylobacter* contamination in retail poultry meat and by-products in the world. The present paper is focussed on the occurrence of *Campylobacter* in retail poultry meat in Europe taking into account the available literature published between 2006 and mid 2016. Afterwards, starting again from the available literature, the authors analyze and discuss the main factors that may affect the spread of *Campylobcter* in retail poultry meat, and the risks related to the presence of this pathogen in the same food matrix.

Case Studies

The literature review that follows was performed based on the PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and ScienceDirect (http://www.sciencedirect.com/) databases using the terms "*Campylobacter* AND retail", "*Campylobacter* AND Europe", "*Campylobacter* AND European Union", "*Campylobacter* AND chicken", "*Campylobacter* AND meat", "*Campylobacter* AND poultry". Restrictions were imposed in order to select only the papers published during the last decade (2006–2016). The reviewed papers were reported based on the year of the studies and not on the year of their publication.

In accordance with the national *Campylobacter* surveillance programme foreseen by the Danish Veterinary and Food Administration, Andersen et al. (2006) carried out a 7-year monitoring (1996–2003) on the occurrence of antimicrobial resistance among *C. jejuni* isolated from raw poultry meat at retail level in Denmark. The poultry meat was brought from national farmers or from other European countries (as imported meat). The samples included raw chicken, duck and turkey meat collected as either whole carcasses or single parts, such as breasts and legs, for a total of 767 samples. Isolates of *C. jejuni* showed resistance to chloramphenicol, ciprofloxacin, erythromycin, nalidixic acid, streptomycin, and tetracycline, with various frequencies. None of the isolate was resistant to gentamicin. The analyses carried out by Andersen et al. (2006) found significant differences among isolates from poultry meat sources regarding their resistance to tetracyclines; in more detail, isolates from duck and turkey meat showed 32% resistance to this class of antibiotics, whereas chicken meat showed 7.6% resistance. Most isolates (80%) were fully sensitive to the tested antimicrobials, whereas just two isolates showed multiresistance.

With the aim to investigate the genetic diversity of *C. coli* and *C. jejuni* isolates and the possible link between the different genotypes found and the source of isolation, Zorman et al. (2006), between 2001 and 2002, carried out the genotyping of *Campylobacter* isolates from retail chicken meat and from clinical specimens in Slovenia and Bosnia-Herzegovina. A high number of *C. coli* strains occurred in poultry samples. Moreover, pulsed-field gel electrophoresis (PFGE) suggested that, due to cross-contamination, transmission of *C. jejuni* and *C. coli* occurred between the chickens on the farm and the retail chicken meat, as well as from the retail meat to humans.

Between 2001 and 2007, Boysen et al. (2011) carried out a retrospective survey analysis on the prevalence of thermotolerant *Campylobacter* species in conventional broiler flocks at slaughter, and broiler meat at retail in Denmark. The authors found a significant effect of season on the prevalence of *Campylobacter* in meat at retail. In more detail, a high prevalence of *Campylobacter* was observed during summer and autumn, whereas a low prevalence was observed during winter and spring. Moreover, the occurrence of *Campylobacter* in broiler flocks, was found to be a good predictor for the presence of *Campylobacter* in fresh and chilled broiler meat.

Between January 2002 and December 2003, Praakle-Amin et al. (2007) studied broiler chicken and turkey *Campylobacter* isolates in samples of meat from Denmark, Estonia, Finland, United States and Hungary, all sold in Estonian retail stores. The majority of the isolates belonged to *C. jejuni*. While isolates showed single and/or multiple resistances to antimicrobial as ciprofloxacin, nalidixic acid, tetracycline, ampicillin, erythromycin (this latter only in isolates from chicken samples), none of the isolates from chicken were resistant to gentamicin, moreover, no isolates from turkey were resistant to erythromycin or gentamicin.

In the year 2006, Soonthornchaikul et al. (2006) published a paper that compared the diffusion of antibiotic resistance in *Campylobacter* isolated from organically- versus intensively-reared chickens purchased from retail outlets in the United Kingdom. Isolates obtained from the different typologies of meat showed different resistance rates to ciprofloxacin, erythromycin and nalidixic acid. In more detail, 100% of chickens harboured *Campylobacter* isolates that were resistant to erythromycin and nalidixic acid, independently from the rearing regime. All of the isolates belonging to the organically-reared group showed to be susceptible to ciprofloxacin, whereas the isolates from intensively reared chickens showed different resistances to this antibiotic. Interestingly, 8.7% of the intensively-reared chickens, sheltered resistant isolates on the skin, thus suggesting a possible post-slaughtering contamination, whereas the highest percentage of resistant isolates (26.7%) was discovered inside the carcasses, thus suggesting a possible link to antimicrobial treatments carried out during rearing.

Between 2006 and 2008, Strachan et al. (2012) carried out a study on the source, prevalence and load of *Campylobacter* spp. in specimens of retail livers (from chicken, cattle, pig and sheep). Among the samples, 81.0% (21 out of 26) of chicken livers revealed the presence of the pathogen. The comparison of the multilocus sequence typing (MLST) genotypes of *Campylobacter* isolated from chicken livers with those of the isolates of human origin showed a great overlap, thus providing further evidence that chicken livers may represent a vehicle of pathogenic *Campylobacter* to humans.

Prencipe et al. (2007) carried out a survey on the contamination levels of *Campylobacter* in marketed poultry meat samples collected in Central Italy. On a total of 392 samples of whole and sectioned chickens, 160 revealed low levels (0.3–9.3) of the pathogen expressed as most probable number (MPN) g^{-1}, whereas 17 and 1 samples revealed levels of *Campylobacter* > 9.3 and of 110 MPN g^{-1}, respectively. The most commonly isolated species belonged to *C. jejuni* (81.9% of the isolates) followed by *C. coli* (32.5%). Moreover, Prencipe et al. (2007) reported a high prevalence of the pathogen in small- and large-scale retail stores, without significant differences in contamination levels depending on product type (loose or packaged) or distribution channels. The authors highlighted the need to implement national surveillance systems and registration procedures for campylobacteriosis.

In 2009, Bardoň et al. (2011) determined the prevalence of thermotolerant *Campylobacter* spp. on broiler chickens (*Gallus gallus*) directly collected in retail stores (supermarkets) in the eight largest cities in the Czech Republic. The study was carried out in accordance with the State Veterinary Administration of the Czech Republic and involved 240 samples (120 chilled and 120 frozen) of poultry meat. A high prevalence (56.0%) of *Campylobacter* spp. in broiler chickens at retail was discovered, moreover, high resistance rates to ampicillin, oxolinic acid and ciprofloxacin were detected for both *C. jejuni* and *C. coli* isolates. None of the isolates were resistant to chloramphenicol or gentamicin.

Following a monitoring plan on chicken meat products in retail outlets carried out from April to December 2009 in France, Guyard-Nicodème et al. (2015) found 76.0% of positive samples for the presence of *Campylobacter* among the 361 chicken products analyzed. As expected, the authors found a significantly heavier contamination on products with skin in respect to those without skin. Among the analyzed chicken meat products on sale, *C. jejuni* was more prevalent than *C. coli*. All the tested isolates were susceptible to chloramphenicol, erythromycin and gentamicin, while they were resistant to ciprofloxacin, nalidixic acid and tetracycline.

C. jejuni and *C. coli* were identified in 51.4% and 37.7% of 312 broiler meat products analyzed in Lithuania from 2009 to 2010 by Kudirkiene et al. (2013). The occurrence and counts of *Campylobacter* spp. varied significantly between the producers examined.

A recent survey carried out in retail poultry meat in the year 2012 in Estonia by Mäesaar et al. (2014) discovered a significant variation due to season effect in the presence of *Campylobacter*, with peaks in the counts during the summer period. Samples included Estonian, Lithuanian and Latvian products, all sold in the Estonian retail market. The mean viable count of *Campylobacter* in the positive samples analyzed was 3.2 Log cfu/g, with a prevalence of *C. jejuni* (89.0%) among the isolates. Samples originating from Latvian and Lithuanian producers showed the highest counts of *Campylobacter*.

To assess the prevalence of *Campylobacter* in poultry meat, Andrzejewska et al. (2015) analyzed a total of 742 poultry meat samples over a five year period (2009–2013). The meat, including chicken and turkey filets, chicken wings, leg quarters, drumsticks, and giblets, was collected in Northern Poland from supermarkets and butcher shops. The results indicated that among the poultry meat samples, 309 (41.6%) were positive for *Campylobacter* spp.; *C. jejuni* was the prevalent species (62.8% of positive samples), whereas *C. coli* was found in 37.2% of the tested products. The assessment of antimicrobial susceptibility of the isolates highlighted different levels of resistance. In more detail, 66.3% of the strains were resistant to ciprofloxacin, 42.3% to tetracycline, 2.6% to erythromycin, and 0.6% to azithromycin. The study of Andrzejewska et al. (2015) confirmed the high level of fluoroquinolone resistance among *Campylobacter* species. These authors also highlighted the need for additional data on the prevalence of *Campylobacter* in poultry meat to determine the more appropriate hygienic measures on farms and control measures during carcass slaughtering, to be used to reduce the occurrence of *Campylobacter* in retail poultry meat.

In 2009, Suzuky and Yamamoto published a literature survey, supported by the Ministry of Health, Labour, and Welfare of Japan, on *Campylobacter* contamination in retail poultry meats and by-products in the world, covering a period of about 6 years (2002–2007). The results showed that retail poultry meat and by-products were mostly contaminated by *C. jejuni* in the majority of the countries, with detection frequencies between 28.1% (South Africa) and 100% (Argentina, Belarus and Russia), whereas *C. coli* was less frequently isolated.

As emerged from the reviewed literature summarized in Table 1, the findings of Suzuky and Yamamoto (2009) on the prevalence of *Campylobacter* species seem to be confirmed, though it is worth noting that the data on the prevalence of *Campylobacter* spp. available in literature are sometimes difficult to compare, for differences in the sampling design or diagnostic methods followed.

In more detail, the majority of the reviewed studies reported that, among positive samples, detection frequencies for *C. jejuni* were comprised between 46.5 and 91.4%, thus clearly confirming this species as the most relevant in the contamination of retail poultry meat. *C. coli* was found at a clearly lesser extent. *Campylobacter* species naturally occur in the intestine of warm-blooded animals, and in particular, birds. Accordingly, the main sources of *Campylobacter* infection are poultry and meat products (Pitkänen, 2013) while wild mammals and birds have been recognized as possible reservoirs for *Campylobacter* transmission to livestock (Sippy et al., 2012). Currently there is no EU legislation regulating the presence of *Campylobacter* in meat. Faeces, foodstuffs, litter and also aerosol are the main sources of *Campylobacter* contamination of poultry.

With regards to the contribution of the retail system in the loads of *Campylobacter* reported in the reviewed literature, it is worth noting that the storage temperature of meat could affect the final counts since it is known that *Campylobacter* is able to replicate at 4°C and at room temperature (Lee et al., 1998). Indeed, as reported by Wei et al. (2016), the initial contamination level of poultry meat can vary depending on post slaughter treatments, temperature control and hygiene management during the processing or storage. As an example, Lundén et al. (2014) reported failures in temperature control efficacy of refrigeration equipment in retail stores in Finland, with temperature abuses observed in 50% of the products. Moreover, Lundén et al. (2014) found that poor practices regarding temperature settings were carried out and that temperature limit was exceeded by over 3°C for more than 30 min in 17.9% of the products.

As reported by Wegener (2010) the requests of the market can also influence the quality of fresh poultry meat sold at retail. Indeed, Wegener (2010) reported that among the controls of *Campylobacter* in broiler chicken meat carried out in Denmark, the *Campylobacter*-positive flocks were usually used for the production of frozen poultry products, whereas fresh poultry products were usually prepared using *Campylobacter*-negative flocks. Notwithstanding, owing to logistical problems, in summer the request for fresh poultry can sometimes exceed the supply of *Campylobacter*-negative flocks.

ORIGIN OF *CAMPYLOBACTER* IN POULTRY MEAT

Farming

The colonization of the intestinal tract in broiler chicks is usually undetectable before the age of 7 days, whereas the overall occurrence of *Campylobacter* within the flock can reach the highest level at the slaughter age. As reported in a recent review on the ecology of *Campylobacter* in poultry, in some chicken flocks the presence of the pathogen can attest up to a frequency of 100% (Sahin et al., 2015). Though the epidemiology of *Campylobacter* in broiler production is still not fully understood, at the present time, the horizontal transmission seems to be responsible for flock colonization since the contribution of vertical transmission from parents to their offspring remains still uncertain (Henry et al., 2011). Indeed, as reported by Sahin et al. (2015) it is well established that the pathogen is rarely detected in flocks of 2–3 weeks of age, whether chickens or turkeys are considered.

Although information on the colonization and ecology of *Campylobacter* in respect of chickens is already available in the scientific literature, there a paucity of reports on possible actions of intervention in order to limit the presence of the pathogen in both farms and meat.

Giombelli and Gloria (2014) have recently reported the presence of *C. jejuni* and *C. coli* in samples of litter, with a prevalence of 100% and 58.8% of the two species, respectively. Sommer et al. (2013) have recently

Table 1. Occurrence of thermotolerant *Campylobacter* species in retail poultry meat in the European Union (1996–2013).

Years	EU Country	Number of samples	Retail typology	Food analyzed	% of positive samples	Species detected	Detection and/or identification methods	AR	Reference
1996-2003	Denmark	767	Retail outlets and wholesalers	Raw chicken, duck and turkey meat (whole birds, breasts and legs)	n.r.	*C. jejuni*	Standard methods, including hydrolysis of hippurate- and indoxyl acetate	Chloramphenicol, erythromycin, ciprofloxacin, nalidixic acid, streptomycin, tetracycline	Andersen et al. (2006)
2001-2002	Slovenia Bosnia and Herzegovina	n.r.	Retail outlets	Poultry meat	n.r.	*C. jejuni, C. coli*	ISO 10272 followed by PCR based on amplification of flagellin genes, hippuricase gene (*C. jejuni*) and aspartokinase gene (*C. coli*)	n.r.	Zorman et al. (2006)
2001-2007	Denmark	n.r.	Retail establishments or wholesale outlets	Fresh chilled and frozen broiler meat	n.r.	n.r.	Selective enrichment followed by culture on a selective media	n.r.	Boysen et al. (2011)
2002-2003	Estonia	610	Retail stores	Broiler chickens and turkeys	n.r.	*C. jejuni* (91.4%), *C. coli* (5.7%), *Campylobacter* spp. (2.9%)	Standards methods followed by serotyping and PFGE genotyping of the isolates	Ciprofloxacin, nalidixic acid, tetracycline, ampicillin, erythromycin (this latter only in chicken samples)	Praakle-Amin et al. (2007)
2005	United Kingdom	90	Supermarkets and butcher's shops in London	Fresh pre-packaged organically-reared and intensively-reared chickens, fresh unpackaged intensively-reared chickens	80.0-100	*Campylobacter* spp.	Standard methods	Erythromycin, nalidixic acid, ciprofloxacin	Soonthornchaikul et al. (2006)
2006-2008	United Kingdom	26	Retail supermarkets and butcher's shops	Chicken, cattle, pig and sheep livers	81.0	*Campylobacter* spp.	Standard methods, genotyping by traditional 7 locus multilocus sequence typing (MLST)	n.r.	Strachan et al. (2012)
2007	Italy	392	Small- and large-scale retail stores	Whole and sectioned chickens	45.4	*C. jejuni* (81.9%), *C. coli* (32.5%)	ISO 10272:95	n.r.	Prencipe et al. (2007)
2009	Czech Republic	240	Supermarkets	Chilled and frozen poultry meat	56.0	*C. jejuni* (70.0%), *C. coli* (18.0%)	ISO 10272-1:2006 ISO 10272-2:2006	Ampicillin, oxolinic acid, ciprofloxacin	Bardoň et al. (2011)
2009	France	361	Retail outlets	Chicken meat products (carcasses, chicken legs and chicken fillets)	76.0	*C. jejuni* (46.5%), *C. coli* (34.9%)	ISO 10272, PCR	Tetracycline, ciprofloxacin, nalidixic acid	Guyard-Nicodème et al. (2015)
2009-2010	Lithuania	312	Retail	Chicken wings and drumsticks	n.r.	C. jejuni 51.4%, C. coli 37.7%	Standards methods followed by *fla*A-RFLP typing	n.r.	Kudirkiene et al. (2013)
2009-2013	Poland	742	Supermarkets and butcher shops	Chicken and turkey filets, chicken wings, leg quarters, drumsticks, and giblets	41.6	*C. jejuni* (62.8%), *C. coli* (37.2%)	ISO 10272-1:2006	Ciprofloxacin, tetracycline, erythromycin, azithromycin	Andrzejewska et al. (2015)
2012	Estonia	606	Retail market	Company-packaged chicken, turkey, laying hen duck meat (as fresh meat, carcasses, minced meat, meat Preparations, heat-treated poultry meat products	20.8	*C. jejuni* (89%), *C. coli* (8%), *Campylobacter* spp. (3%)	ISO 10272-1:2006, Multiplex PCR	n.r.	Mäesaar et al. (2014)

n.r. not reported.
PCR Polymerase Chain Reaction.
RFLP Restriction Fragment Length Polymorphism.

summarized the factors that can be significantly associated with the *Campylobacter* status of the flocks, among these: age of broiler house, rodent control, age of broiler at slaughter, number of broiler houses on the farm, etc. Sahin et al. (2015) also summarized the main control measures (pre- and post-harvest) useful in poultry farming. Among the pre-harvest measures, it emerged that the careful application of good hygiene practices (staff training, rodent control, drinking water decontamination, etc.) are important tools to avoid, or limit, *Campylobacter* entrance in the broiler farm, although their implementation undoubtedly involves additional costs. Among the above cited measures, drinking water acidification (e.g. with organic acids as lactic acid) proved to be able to reduce the risk of colonization of the flocks (Allain et al., 2014). Acidification of the litter carried out with aluminium sulphate and sodium bisulphate was also able to reduce the contamination of the flocks (Line, 2002), whereas the acidification of the feed was successfully used as a further control measure against *Campylobacter* eventually present in the gut of chickens. On the contrary, the use of bacteriophages and immune interventions proved to be ineffective (Sahin et al., 2015).

Very recently, Torralbo et al. (2014) carried out a large epidemiological study to determine the occurrence of *Campylobacter* infection in broiler farms in Spain, in relation to different risk factors. The authors identified a series of factors that may affect the contamination level of *Campylobacter* in a negative way, among which: the presence of pets on the farm, the age and the application of thinning of broiler flock, the presence of canvas blinds at the windows, and the occurrence of rodents in the poultry house.

Slaughtering

The presence of *Campylobacter* in the intestinal tract can lead to carcass contamination during slaughtering, moreover, it is known that defeathering can increase contamination. Among post-harvest control measures, scalding and chilling of the carcasses proved to be able to reduce the contamination level of *Campylobacter*. The removal of faecal residues followed by the use of chlorine-based antimicrobial were also useful. In regards to this issue, it is worth noting that the chemical decontamination of carcasses, allowed in the United States, is prohibited in Europe. The analysis of the data reviewed by Sahin et al. (2015) also found that the systematic analysis of critical control points along the slaughter process helps to identify the most appropriate measures to control contamination of carcasses.

Melo et al. (2013) have evaluated the virulence characteristics of 55 *C. jejuni* strains isolated from chicken carcasses, highlighting the potential role of these strains in the pathogenesis of human disease. Melo et al. (2013) have also recommended the enhancement of vigilance and strict controls during production as measures to protect the health of consumers.

Many studies reported the influence of slaughtering on the contamination of carcasses (Stern and Robach, 2003; Reich et al., 2008). Indeed, some authors argued that the effectiveness of the controls carried out in slaughterhouse to reduce the incidence of human campylobacteriosis is higher by about 30 times compared to the biosecurity measures in the herd (Rosenquist et al., 2003). In some countries, such as Denmark, interventions for the control of campylobacteriosis have been directed to the reduction of *Campylobacter* spp. in broiler flocks, as well as to the activities carried out at the slaughterhouse (Wagenaar et al., 2006).

In order to prevent potentially contaminated meat from entering the market, Josefsen et al. (2015) proposed a cost-effective approach based on on-site or at-line monitoring at slaughterhouses by using the same detection platform available for *Salmonella* or other food-borne pathogens. Josefsen et al. (2015) also proposed the use of culture-independet metagenomic-based techniques that allow a single-step detection and identification of *Campylobacter*.

ANTIBIOTIC RESISTANCE

Last but not least, as the issue of (transferable) antibiotic resistance(s) in *Campylobacter* is concerned, the resistance to certain antibiotics developed by *Campylobacter* is of particular importance in the food chain because such phenomenon inevitably has an impact on the treatment of human infections. It is worth noting that the incidence of resistance to several important antibiotics used in the treatment of this disease is increasing. Moreover, the presence of multiple resistances due to the (mis)use of antibiotics in veterinary medicine and breeding, with the consequent diffusion of transmissible antibiotic resistance genes in the food chain, mediated by pro-technological microorganisms (e.g. lactic acid bacteria) (Aquilanti et al., 2007; Clementi and Aquilanti, 2011), must be considered. This alarming emergence is a further factor of risk for the health of consumers that should deserve to be carefully monitored.

As recently reported by Wieczorek and Osek (2013), *Campylobacter* showed to be resistant to fluoroquinolones, macrolides, aminoglycosides, and betalactams. In addition, intrinsic resistance of *C. jejuni* and *C. coli* to penicillins and most cephalosporins, as well as to rifampicin, sulfamethoxazole, trimethoprim, and vancomycin was described.

As emerged from the reviewed literature, the most reported antibiotic resistances in *Campylobacter* isolates from poultry meat at retail were to tetracycline, ciprofloxacin, and nalidixic acid whereas resistance to gentamicin was never observed.

The analysis of the reviewed literature is basically in line with the results reported in the EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014 (EFSA and ECDC, 2015a). In more detail, the EU summary reported that a high to very high proportion of isolates of *Campylobacter* from humans were found to be resistant to ciprofloxacin and tetracyclines, whereas resistance to erythromycin was from low to moderate. Moreover, resistance to fluoroquinolones in some MSs was extremely high. Regarding *C. jejuni* isolates from broilers and broiler meat, high resistance to ciprofloxacin (69.8%), nalidixic acid (65.1%) and tetracycline (54.4%) was reported, whereas much lower levels of resistance were recorded for erythromycin (5.9%) and gentamicin (0.9%). A similar pattern of resistance to these substances occurred in *C. coli* from broilers. Over the 2008–2014 period, resistance to ciprofloxacin, erythromycin and nalidixic acid in broilers greatly varied in the different MSs under review; in several cases statistically significant increasing trends were observed in the resistance to the above cited antimicrobials, for both *C. jejuni* and *C. c*oli (EFSA and ECDC, 2015a).

The above findings shed increasing concern on the effectiveness of antibiotic treatments for human *Campylobacter* infections in a near future.

CONCLUSIONS

Currently, the European legislation does not include any law foreseeing specific criteria related to the presence of *Campylobacter* in food. Therefore, pending the filling of this gap, some voluntary preventive measures are desirable in order to limit the occurrence of the pathogen especially in the poultry meat industry. Though the fundamental risk factors involved in the contamination and growth of *Campylobacter* in poultry meat are known, more structured information is needed on the preventive actions that could be implemented at breeding, slaughtering, processing and retail. Indeed, each of these steps have a not negligible role in the dissemination of *Campylobacter* and should therefore be addressed through properly implemented good hygiene practices.

The further issue linked to *Campylobacter* dissemination, that is the increasing human exposure to antibiotic resistant bacteria, must be urgently addressed through adequate measures. Prevention of the diffusion of antibiotic resistant *Campylobacter* should begin during rearing and should involve an use as restricted as possible of all the antibiotics, with particular attention to those critically important to human medicine.

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