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Prevalence and quantification of thermophilic *Campylobacter* spp. in Italian retail poultry meat: Analysis of influencing factors

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ABSTRACT

Retail poultry meat is a crucial vehicle for consumers' exposure to Campylobacters, but no official controls are currently applied in Italy. The aim of this study was the evaluation of *Campylobacter* contamination of a wide range of poultry meats marketed in Italy. N. 472 chicken and turkey meat samples (sectioned meats, offal, meat preparations and products) were taken from slaughterhouses, deboning plants and different retailers and submitted to detection/enumeration of *Campylobacter* spp. The isolates were identified by phenotypic and biomolecular techniques. *Campylobacter* spp. was detected in 34.1% of the samples, with general low counts. Higher values were observed in offal (especially liver) and sectioned meats, with significantly higher rates in skin-on samples (86.8% *vs* 32.7%). Minced meat preparations showed lower prevalence (22.4% *vs* 58.3%) and counts than whole pieces. Decreasing rates were observed among slaughterhouses (80%), deboning plants (49%), butcher's shops (37%) and large scale retailers (25%). Sectioned chicken meats were significantly more contaminated than turkey meats. Almost all the isolates were identified as *C. jejuni* or *C. coli*, with similar prevalences (18.4% and 20.5%, respectively); *C. jejuni* was predominant only in samples from slaughterhouses/deboning plants. For setting future control programs, meat typology should be considered the main critical factor.

1. Introduction

Campylobacteriosis is by far the most common foodborne infection in the European Union, with more than 200.000 confirmed human cases/year (EFSA-ECDC, 2015). Data from Italy show a lower prevalence of human infections than other European countries, but it's known that the available data are underestimated, due to underreporting of mild cases and to the absence of an official monitoring programme.

The role of poultry as a reservoir for the transmission of *Campylobacter* to humans has already been recognized, with 20–30% of the human infections linked to handling, preparation, and consumption of broiler meat (EFSA, 2010b). It's known that the risk posed by poultry meat is strongly associated to the presence of high *Campylobacter* loads rather than to its diffusion (EFSA, 2009, 2011; Nauta et al., 2009), and guidelines supplied by EFSA for the harmonized control of Campylobacters in poultry meat underline the importance of a quantitative approach (EFSA, 2008).

With the aim of assessing the exposure to the *Campylobacter*-associated risk, the evaluation of poultry meats at retail is critical, as they really enter the consumers' kitchens (Cook et al., 2012). It's known that *Campylobacter* spp. is strongly affected by the environmental conditions, such as oxidative stress, osmotic shock and

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drying, resulting in a gradual inactivation during production and storage. So, also if a diffusion of the contamination due to cut and manipulation can happen, lower positivity rates and after all lower counts are often detected at processing level or at retail than earlier in the production chain (Berrang et al., 2001; Uyttendaele et al., 1999). On the other hand, the modern, short processing chain, and the use of protective plastics and dark, moist and cool storage contribute to *Campylobacter* survival, especially in large scale marketing (EFSA, 2009; Harrison et al., 2001). So, high prevalences at retail (60–80%) are sometimes detected, and the presence of some heavily contaminated (>10^4 CFU/g) meat cuts and preparations have been reported by several authors (Humphrey et al., 2007; Suzuki and Yamamoto, 2009; Uyttendaele et al., 2006).

Significant differences can be detected among different typologies of poultry meat available on the market, with a decreasing trend from whole carcasses to parts, especially when skin is removed. Due to the inhibitory activity of additives and to the oxidative stress, low frequencies and counts are reported for minced meat and meat preparations, while meat products are considered substantially safe (Cook et al., 2012; Habib et al., 2008; Mena et al., 2008; Meldrum et al., 2006; Uyttendaele et al., 1999, 2006). A particular case is represented by offals (especially livers), that often carry high microbial numbers, leading in some cases to campylobacteriosis outbreaks (Baumgartner et al., 1995; Little et al., 2010; Whyte et al., 2006).

Considering the diffusion of *Campylobacter* species, it's known that almost all the isolates coming from poultry meat in Europe belong to *C. jejuni* and *C. coli*, with a general rate of 2/3 and 1/3 of the isolates, respectively, but this ratio varies among countries, and tends to reach a 1/1 value in Southern Europe (EFSA, 2010a; Suzuki and Yamamoto, 2009). Some data suggest the presence of a higher resistance of *C. coli* to environmental conditions, resulting in a relatively higher prevalence of this species in processed meats (Padungtod and Kaneene, 2005).

Official Italian data concerning the prevalence of *Campylobacter* spp. on poultry meats at retail are lacking; the last available studies indicate a variable situation (Nobile et al., 2013; Sammarco et al., 2010). The aim of this study was to collect qualitative and quantitative data concerning the contamination by thermophilic Campylobacters on a wide range of poultry meats marketed in Italy, evaluating the main factors influencing their prevalence and loads.

2. Materials and methods

2.1. Samples selection and experimental design

A total of 472 chicken and turkey meat samples (353 samples of chicken meats, 83 of turkey meats and 36 of mixed meats), including raw sectioned meats, meat preparations and products were submitted to detection and enumeration of *Campylobacter* spp. The samples were obtained from various slaughterhouses, deboning plants and retail sales, located in different Italian regions (Lombardy, Veneto, Tuscany and Sicily) during the period September 2010–June 2013. All the samples were portioned and packed for the retail market. The sampling plan is showed in Table 1.

2.2. Sampling and microbiological analyses

The samples were withdrawn on the day of preparation for sale/distribution. Each sample was put into sterile stomacher bags (BagLight, Interscience, Saint Nom, F) and transferred to the laboratories, where the analyses were performed within the same day.

For the detection of *Campylobacter* spp., the EN ISO 10272-1:2006 method was applied. As requested by the ISO method, the inoculation of mCCDA agar plates (Oxoid, Basingstoke, UK), was combined with another method based on a different principle. The method described by Steele and McDermott (1984), with some modifications, was chosen. An aliquot of 0.5 ml of the enrichment broth was put onto 47 mm diameter, 0.45 μm pore size cellulose membrane filters (Sigma Aldrich Italy, Milan, I) laid on the surface of non-selective blood agar plates (Columbia Agar base added with 5% of defibrinated sheep blood, Oxoid). The membranes were left for 45 min and then removed, taking care to avoid the spilling of the broth; the filtered inoculum was spread on the surface by a sterile 10 μl loop. The plates were then incubated at 42 °C for 48 h. Typical colonies were isolated by subculturing on Columbia-blood agar and submitted to further confirmation steps.

The enumeration of *Campylobacter* spp. in the samples was performed on mCCDA plates by the EN ISO 10272-2:2006 method. For each sample, 5 colonies (when present) were picked, subcultured on blood agar plates incubated 41,5 °C for 24–48 h in microaerobiosis and submitted to confirmation/identification tests.

2.3. Identification of the isolates

For the identification of the isolates, further steps were performed. Cells morphology and motility were evaluated by microscope observation (1000× magnification) of a suspension of the isolates in 1 ml of Brucella broth (Oxoid); oxidase determination (Oxidase strips, Oxoid) and Gram staining were also performed. The isolates were then subcultured in two series of blood agar plates, one incubated at 41.5 °C for 44 ± 4 h in aerobiosis, and the other incubated at 25 °C for 44 ± 4 h in microaerobic atmosphere. For the provisional identification of the species, the isolates showing the typical characteristics of thermophilic *Campylobacter* spp. (little curve Gram negative rods with corkscrew motility, unable to grow in aerobiosis or at 25 °C, oxidase positive) were evaluated for the catalase activity and the susceptibility to cephalotin (30 μg) and nalidixic acid (30 μg) (Oxoid) by the disk diffusion method, and were submitted to the identification by API Campy kit (bioMerieux Italia, Bagno a Ripoli, I).

In the further identification step, the isolates were prepared, properly labelled and sent from each research unit to a unique laboratory for the biomolecular identification. The strains were inoculated onto blood agar plates and incubated at 41,5 ± 1 °C for 48 h in microaerobic conditions; bacterial slime was recovered by a sterile swab, that was inserted into a tube of Amies transport medium with charcoal (Oxoid). For DNA extraction, the strains were also inoculated in Brain Heart Infusion broth (BHI, Oxoid) added with 5% of laked horse blood and 15% of glycerol, and stored at −80 °C until the analysis. For the identification of the genera (*Campylobacter, Helycobacter, Arcobacter*), a simplex PCR-RFLP analysis of the 16S rRNA Gene was performed, following the method described by Marshall et al. (1999). The isolates identified as *Campylobacter* spp. where then submitted to species identification by a multiplex PCR method, as described by Wang et al. (2002).

2.4. Statistical analysis

All the data obtained from qualitative analyses were submitted to the frequency distribution analysis (χ square test) considering the following factors: sample typology, supplier typology, meat species and sampling season. The differences among the counts obtained considering the same factors were also evaluated, using the SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC). A value of $P < 0.05$ was considered statistically significant.