



## Prevalence of thermotolerant *Campylobacter* spp. in broilers at retail in the Czech Republic and their antibiotic resistance

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### ARTICLE INFO

#### Article history:

Received 1 March 2010

Received in revised form

26 July 2010

Accepted 3 August 2010

#### Keywords:

*Campylobacter* spp.

Prevalence

Antibiotic resistance

Poultry

Retail

### ABSTRACT

Infections caused by thermotolerant *Campylobacter* spp. are among the most frequent bacterial diseases from food in Europe. An important source of infection is poultry. The aim was to perform the first nationwide study on the prevalence of *Campylobacter* spp. at retail in the Czech Republic. Samples of chilled and frozen broiler chickens were obtained from supermarkets localized in the eight largest cities in the Czech Republic. From February to November 2009, a total of 120 samples of chilled and 120 samples of frozen poultry were collected and assessed. Qualitative and quantitative analyses were performed according to the ISO 10272-1:2006 and ISO 10272-2:2006 methods. The microdilution method was used to test the resistance of *Campylobacter* spp. isolates to selected antibiotics. High prevalence of *Campylobacter* spp. was detected in chilled poultry (75%). The prevalence was significantly lower (37%) in frozen poultry. The most prevalent species was *Campylobacter jejuni* detected in 70% of positive findings, followed by *Campylobacter coli* (18%). In 12% of cases, the samples were contaminated by both *C. jejuni* and *C. coli*. The species *Campylobacter lari* and *Campylobacter upsaliensis* were not detected. The highest resistance rates were to quinolone antibiotics and ampicillin.

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### 1. Introduction

Thermotolerant species of the *Campylobacter* genus, in particular *Campylobacter jejuni* and *Campylobacter coli*, are the most common bacterial pathogens causing human digestive tract infections in the Czech Republic and many other European countries (EFSA, 2009; EPIDAT, 2010). In 2009, the incidence of gastrointestinal tract diseases due to *Campylobacter* spp. reached 195 cases per 100,000 population in the Czech Republic. For comparison, the incidence of gastrointestinal infections caused by *Salmonella* spp. was only 103 cases per 100,000 (EPIDAT, 2010). In 2007, *Campylobacter* spp. were the most frequently reported cause of bacterial gastrointestinal infections in the EU. The number of confirmed cases in the EU rose from 175,564 in 2006–200,507 in 2007 (EFSA, 2009). In 2007, the most commonly detected species was *C. jejuni* (44%), followed by *C. coli* (3%); the other species accounted for a total of 7%. In the remaining 46% of isolates, species were not identified. The most affected group in Europe were children under

4 years of age (EFSA, 2009). There is also a high incidence of *Campylobacter* infections outside Europe (Chrystal, Hargraves, Boa, & Ironside, 2008).

An important source of infection is poultry meat. Gormley et al. (2008) suggested that humans were at a considerable risk of *Campylobacter* infection from poultry. Therefore, a number of European countries monitor the prevalence of *Campylobacter* spp. directly at retail. In 2007, the prevalence of *Campylobacter* spp. in the Austrian retail chain was 63%. The studies detected *Campylobacter* spp. in 74% of 162 samples of chilled poultry and in 31% of 57 samples of frozen products (EFSA, 2009). In a one-year study carried out in Northern Ireland, Moran, Scates, and Madden (2009) found prevalence rates of 91% in chicken, 56% in turkey and 100% in duck. The most prevalent isolates were those of *C. jejuni* (65%), followed by *C. coli* (27%) and *Campylobacter lari* (1%). A similar study was performed by Meldrum and Wilson (2007) in Wales and Northern Ireland between March and December 2005. The study focused on the prevalence of *Campylobacter* and *Salmonella* spp. in raw chicken at retail. Of 877 investigated samples, *Campylobacter* and *Salmonella* spp. were present in 70% and 4%, respectively. The authors reported a significant difference between chilled and frozen poultry meat, with higher prevalence rates in chilled meat products (Meldrum & Wilson, 2007). According to Suzuki and

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Yamamoto (2009), the prevalence of *Campylobacter* spp. in poultry at retail in Europe exceeds 50%, the exceptions being Estonia and Belgium with prevalence rates of 8% and 17%, respectively. The authors found *C. jejuni* to be the most prevalent species both in and outside Europe, with the exception of Thailand and South Africa where *C. coli* was most common in poultry at retail.

To assess the risk of alimentary infections, the numbers *Campylobacter* spp. bacteria are worth studying. In Germany, contamination of the surface of chicken legs at retail was tested by Scherer, Bartelt, Sommerfeld, and Hildebrandt (2006). Enumeration of *Campylobacter* spp. on the surface of positive chicken legs revealed a median of 2.4 log CFU/g.

In their study, Bhaduri and Cottrell (2004) pointed to the fact that chilling and freezing (individually or in combination) resulted in only small decreases in viable counts of *Campylobacter* spp. and therefore are not a substitute for safe handling and proper cooking of raw poultry. Bostan, Aydin, and Ang (2009) studied the prevalence of thermotolerant *Campylobacter* spp. in beef, mutton and chicken at retail in Istanbul, Turkey. *Campylobacter* spp. were detected in 50% of the chicken meat samples. The authors found no significant seasonal changes in the prevalence. The most prevalent species were *C. jejuni* in chicken and *C. coli* in beef and mutton.

Bostan et al. (2009) tested the resistance of 246 *Campylobacter* spp. isolates to selected antibiotics. The isolates were most frequently resistant to tetracycline (69%), trimethoprim–sulfamethoxazole (64%), nalidixic acid (58%) and erythromycin (56%), followed by enrofloxacin (48%), ciprofloxacin (42%), chloramphenicol (36%) and gentamicin (26%). Valenza, Frosch, and Abele-Horn (2010) confirmed that macrolides (erythromycin) should be used as the drug of choice if *Campylobacter* infection is to be treated with antibiotics. Of 113 clinical isolates of *Campylobacter* spp. (105 *C. jejuni*, 7 *C. coli*, 1 *C. lari*), none were resistant to either erythromycin or meropenem, 52% of isolates were resistant to ciprofloxacin and 38% to doxycycline. According to the European Food Safety Authority data, the resistance of *Campylobacter* spp. isolated from poultry meat to erythromycin ranged in Europe from 2% (Hungary) to 12% (Germany) in 2006 (EFSA, 2007a).

Until 2009, no nationwide study on the prevalence of *Campylobacter* spp. in poultry at retail had been performed in the Czech Republic. Therefore, the presented study aimed at determining the prevalence of thermotolerant *Campylobacter* spp. on the skin of chilled and frozen broiler chickens (*Gallus gallus*) directly in retail stores (supermarkets) in the eight largest cities in the Czech Republic.

## 2. Material and methods

### 2.1. Sample collection

The study was carried out from February 2009 to November 2009 according to the State Veterinary Administration of the Czech Republic Guideline No. 1/2009. In the eight largest cities of the Czech Republic, with populations ranging from 95,000 to 1,200,000, supermarkets supplying a significant proportion of residents and offering chilled and frozen poultry meat were selected. From each supermarket, chilled and frozen whole broiler chickens were obtained once a month. Chilled and frozen broilers, all supplied by various domestic producers, were sampled directly as packed for consumers.

A total of 240 samples from throughout the Czech Republic were tested (120 from chilled and 120 from frozen poultry meat). Each month, the same numbers of samples were collected – 12 frozen and 12 chilled chickens. In the largest city (the capital with a population over 1,000,000), 3 chilled and 3 frozen chickens were sampled each month. In two cities with populations exceeding 300,000, 2 chilled and 2 frozen samples were taken each month. In

the remaining 5 cities with populations from 95,000 to 160,000, one chilled and one frozen chicken were sampled each month. Using a refrigerated vehicle, the samples were transported to the laboratory within 24 h from collection and stored there at refrigerator temperature. All samples were processed no later than 24 h after delivery to the laboratory. All samples were collected and subsequently tested in the laboratory prior to the best before date shown on the packaging.

### 2.2. Qualitative and quantitative bacteriological analyses

In the laboratory, skin without fat was removed from the lateral aspect of the chicken neck using aseptic techniques to obtain 27 g of material for further processing. Laboratory analyses to detect thermotolerant *Campylobacter* spp. were performed in accordance with the ISO 10272-1, 2006 standard (qualitative analysis). Enumeration of *Campylobacter* spp. on the skin of broiler chickens was carried out according to ISO 10272-2:2006 (quantitative analysis).

### 2.3. Confirmation and species identification of isolates

To confirm suspect isolates and identify the species, polymerase chain reaction (PCR) methods described by Ertas et al. and Lund et al. (Ertas, Cetinkaya, Muz, & Ongor, 2002; Lund, Nordentoft, Pedersen, & Madsen, 2004) and a commercially available real-time PCR kit (Taq Man *Campylobacter* spp. Kit, AB Applied Biosystems) were applied. For quality control, the *C. jejuni* ATCC 33560 reference strain was used.

### 2.4. Antibiotic resistance tests

In confirmed isolates of *C. jejuni* a *C. coli*, resistance to selected antimicrobial agents was tested by the broth microdilution method (McDermott, Bodeis-Jones, Fritsche, Jones, & Walker, 2005). Testing was performed with prepared frozen microplates containing serial twofold antibiotic dilutions in Mueller–Hinton broth with 2.5% lysed horse blood. The inoculated plates were incubated in a microaerophilic atmosphere (GENbox microaer – bioMérieux) for 48 h at 37 °C. Resistance to 8 selected antibiotics (ATB) was tested. The first 5 ATB (erythromycin, ciprofloxacin, tetracycline, streptomycin and gentamicin) are specified in Commission Decision 2007/516/EC for monitoring of antibiotic resistance in poultry. The advised optimum concentration ranges to be tested for each ATB in mg/L were recommended by the EFSA Journal (EFSA, 2007b). The cut-off values (mg/L) for these ATB were also specified by the above-mentioned Commission Decision 2007/516/EC. Beyond the scope of the EC recommendations, chloramphenicol, ampicillin and oxolinic acid were tested as well. In these three ATB not stated in the decision, the cut-off values (mg/L) were based on the parameters published by the French Society for Microbiology National Breakpoint Committee in 2005 (Communique, 2005). The used ATB, including their parameters, are listed in Table 1. Quality control was performed at regular intervals with the *C. jejuni* ATCC 33560 reference strain.

### 2.5. Statistical analysis

The prevalence of *Campylobacter* spp. in chilled and frozen poultry was statistically analyzed using Student's *t*-test. Differences in the prevalence of *Campylobacter* spp. in individual quarters of 2009 were compared by the ANOVA method (analysis of variance).

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