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Campylobacter species and their antimicrobial resistance in Latvian broiler chicken production

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ABSTRACT

In the present study *Campylobacter* species and their antimicrobial resistance in Latvian broiler chicken production was determined. Furthermore, this is the first report on the antimicrobial resistance patterns for *Campylobacter* isolates from broiler chickens at slaughterhouse and retail level in Latvia. Two biggest Latvian broiler chicken meat producing company products were included in the study. Altogether, 74 randomly selected broiler chicken *Campylobacter* spp. isolates were analysed for species identification. *Campylobacter* isolates were obtained during a 12-month period within the Latvian *Campylobacter* prevalence study in 2010. Colony multiplex PCR was used for all isolates to identify *Campylobacter* species. Minimal inhibitory concentration (MIC) was determined for 58 *Campylobacter* spp. isolates. Resistance to one or more antimicrobials was detected in all 58 isolates (100%). A high proportion of the isolates were resistant to ciprofloxacin (100%) and nalidixic acid (87.9%). Multidrug resistance, which was determined as resistance to three or more unrelated antimicrobials, was detected in 39 isolates (67.2%). Moreover, all multiresistant isolates were resistant to ciprofloxacin and nalidixic acid. Analyses of *Campylobacter* isolates from two Latvian broiler chicken meat producing companies resulted with significant differences in *Campylobacter* species; from the company A mainly *Campylobacter coli were* found, while in the company B *Campylobacter jejuni*.

1. Introduction

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp. From witch *Campylobacter jejuni* and *Campylobacter coli* are the most commonly reported species associated with human intestinal infections in European Union (EU). In average, 55.49 confirmed campylobacteriosis cases per 100,000 EU inhabitants were reported in 2012 (EFSA, 2014). In most cases *Campylobacter* spp. cause gastroenteritis in humans, but may also result in post-infection complications such as Miller–Fisher and Guillain-Barré syndrome (Fica et al., 2011; Kuwabara, 2011). In severe campylobacteriosis cases the antibiotic may be needed to treat the disease, but as the resistance of *Campylobacter* against antibiotics is increasing, the success of the treatment may be compromised (EFSA, 2012; Lehtopolku et al., 2010).

It is well documented that fluoroquinolone resistance among *Campylobacter* isolates from human infections increased after the approval of fluoroquinolones in agriculture, especially in poultry farming (Jacobs-Reitsma, Kan, & Bolder, 1994; McDermott et al., 2002).

The most important source of *Campylobacter* is poultry meat (Friedman et al., 2004). Therefore, the effective control of *Campylobacter* at the farm level and control of the antimicrobial resistance in poultry meat production chain is the major public health strategy (EFSA, 2011). In 2010, the proportions of *Campylobacter*-positive broiler meat samples varied widely in EU member states, from 3.1% to 90%. In accordance with EU-wide

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baseline survey, the average *Campylobacter* prevalence for fresh broiler chicken carcasses was 75.8% (EFSA, 2012). The prevalence of *Campylobacter* in Latvian broiler chicken samples in 2010 was 59.2% (Kovaļenko, Roasto, Liepiņš, Mäesaar, & Hörman, 2013).

The aim of the present study was to determine the *Campylobacter* species and their antimicrobial resistance in broiler chicken production at slaughterhouse and retail level in Latvia.

2. Sampling

A total of 240 fresh broiler chicken neck skin, 240 fresh broiler chicken carcass samples and 2400 whole broiler chicken intact intestines were collected during 2010. Samples were collected monthly at a random basis among products of two biggest companies in Latvia, representing the production of more than 75% of all commercial broilers in Latvia.

Every month, 10 neck skin samples were collected from each of the two investigated slaughterhouse. The neck skin samples were placed separately in sterile plastic bags for transportation to the laboratory. Additionally, every month 10 fresh broiler chicken carcasses from the production of the same broiler meat producers were collected at retail level in Latvia from two biggest supermarket chains. All broiler carcasses were packed individually in plastic bags prior shipping to stores. Carcass samples were collected at the same day as the sampling in slaughterhouses was performed, but they did not represent the same slaughter batch as the neck skin samples and cecal samples. Broiler chicken carcasses from slaughterhouse A were sold in tight, sealed plastic bags whereas from slaughterhouse B broiler chicken carcasses were sold in lose, unsealed plastic bags. Every month, 100 intact broiler chicken intestines were collected from the production of the same broiler meat producers, at the time of evisceration. All 100 samples were divided in ten pooled samples and placed in a single sterile plastic bag for each pooled sample.

All the samples were placed in a portable cooler at a temperature 4–6 °C and transported to the laboratory. Microbiological analyses were carried out immediately after the arrival (2–4 h after sampling) of the samples to the laboratory.

2.1. Isolation and identification of Campylobacter spp.

The isolation of *Campylobacter* was carried out at the Food Hygiene laboratory of the Institute of Food and Environmental Hygiene, Latvian University of Agriculture (Jelgava, Latvia). Immediately after transport, 10 g of sample material, either neck skin or back skin of the broiler chicken carcass, was aseptically taken and placed into sterile plastic bag for enrichment. Plastic bag were then filled with 90 mL of sterile Bolton broth (Oxoid; Basingstoke, Hampshire, UK), and the samples were processed for 1 min in a stomacher. The enrichment broth was then incubated under microaerobic conditions at 37 ± 1 °C for 4 h–6 h, followed by incubation at 41.5 ± 0.5 °C for 44 ± 4 h. After enrichment, 10 μL of the enrichment broth was plated on mCCDA agar (Oxoid; Basingstoke, Hampshire, England) and incubated for 48 h at 41.5 ± 0.5 °C under microaerobic conditions. Typical *Campylobacter* colonies on the mCCDA plates were streaked on the Columbia blood agar (Oxoid) plates, which were incubated for 24 h at 41.5 ± 0.5 °C in microaerobic conditions using anaerobic jars and CampyGen™ sachets (Oxoid). After transportation to the laboratory 10 intestines caeca were dissected and caecal material from 10 caeca was pooled together for one composite sample and 1 g of the content was further analysed. All the analyses and confirmation tests were performed in accordance with instructions of the detection method described by ISO 10272-1:2006 standard. The bacteria isolates that showed typical growth on mCCDA, were gram negative, had corkscrew-like darting motility, were oxidase positive and did not show growth at 41.5 ± 0.5 °C in aerobic conditions or growth at 25 °C in microaerobic conditions, were considered as *Campylobacter* spp.

The *Campylobacter* isolates after isolation were placed into Brain heart infusion broth (Oxoid; Basingstoke, Hampshire, England) with 5% glycerol and stored at −70 °C temperature for further investigation.

From total of 502 *Campylobacter* isolates randomly 74 isolates were selected for *Campylobacter* species identification. All isolates were tested with colony multiplex PCR according to the Wang et al., 2002. By PCR 23s rRNA, hyp O, gly A genes were determined. After thawing and incubation, deoxyribonucleic acid (DNA) extraction and PCR was performed followed by the electrophoresis in agarose gel for electrophoretic evaluation.

2.2. Determination of antimicrobial susceptibility

The determination of antimicrobial resistance was performed for 28 *C. jejuni* and 30 *C. coli* isolates. Only broiler chicken meat origin isolates were studied for antimicrobial susceptibility. These isolates were tested for the minimal inhibitory concentration (MIC) by a broth microdilution method (National Veterinary Institute, Uppsala, Sweden) against erythromycin, ciprofloxacin, tetracycline, gentamicin, streptomycin and nalidixic acid. After frozen storage at −70 °C the *Campylobacter* isolates were cultured on Columbia blood agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 41.5 ± 0.5 °C for 48 h. After incubation, a loopful (1 μl) of bacterial growth was transferred to 2 ml of 0.9% saline and then 100 μl of suspension were added to 10 ml of cation-adjusted Mueller-Hinton broth (CAMHB, Oxoid, Basingstoke, Hampshire, England) with 2.5–5% lysed horse blood to get final inoculum of 10^6 CFU/ml. Each well of MIC panel (VetMIC Camp Ver.2, SVA, Uppsala, Sweden) was inoculated with 100 μl of 10^6 CFU/ml bacterial suspension. After filling of the panel, 10 μl of inoculums were streaked to Columbia blood agar plates for purity control. The density of the bacterial suspension was controlled and colony counts from 50 to 250 per plate were accepted. *C. jejuni* ATCC 33560 was used as a control strain. The plates were incubated at 37 ± 1 °C for 40–48 h in microaerobic conditions. After incubation the MIC was read as the lowest concentration completely inhibiting visible growth of *Campylobacter* in accordance with the instructions given by the test manufacturer (SVA, Uppsala, Sweden).

According to the Eucast (2014a) epidemiological cut-off values *C. jejuni* was considered to be resistant when the MIC values were for: erythromycin >4 μg/ml, ciprofloxacin >1 μg/ml, tetracycline >2 μg/ml, streptomycin >2 μg/ml, nalidixic acid >16 μg/ml and gentamicin >1 μg/ml.

According to Eucast (2014b) epidemiological cut-off values *C. coli* was considered to be resistant when the MIC values were for: erythromycin >16 μg/ml, ciprofloxacin >1 μg/ml, tetracycline >2 μg/ml, streptomycin >4 μg/ml, nalidixic acid >32 μg/ml and gentamicin >2 μg/ml.

2.3. Statistical analysis

Statistical analysis were performed with the Statistical Package R in order to determine statistically significant differences at 95% confidence level in the antimicrobial resistance and species of the *Campylobacter* spp. samples between the two slaughterhouses and between the antimicrobials by Pearson correlation test.